Eid *et al.*
Future Journal of Pharmaceutical Sciences (2024) 10:69
https://doi.org/10.1186/s43094-024-00642-0

Future Journal of Pharmaceutical Sciences

RESEARCH Open Access

Evaluation of antioxidant activity in different Egyptian barley cultivars: an in vitro and in silico study

Omneya Eid[1], Shahira Ezzat[2,3*], Wafaa M. Elkady[1], Abeer El Sayed[2] and Essam Abd el-sattar[2]

Abstract

Background Cereals have historically played a crucial role in the human diet, serving as a significant natural source of energy and offering various health benefits. Barley (*Hordeum vulgare* L.) has been given significant attention in recent years due to its exceptional nutritional value, surpassing that of other cereals. The objective of this research is to evaluate the antioxidant activity of various solvent extracts obtained from three different barley cultivars.

Results The G.136 variety's acetone extract exhibited the highest level of antioxidant activity in both the DPPH assay, with an IC_{50} of 55.62 µg/ml, and the FRAP assay, with 447 µM trolox/mg extract. The dominant compounds identified before in the acetone fraction were subjected to an evaluation of their docking scores, along with an assessment of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology) studies. Notably, hordatine A1, prodelphinidin B3, hordatine B1, procyanidin B2, and isovitexin 7-*O*-glucoside were the major compounds with the highest LipDock scores compared to trolox the reference drug with polyphenol oxidase.

Conclusions The findings indicate that the acetone extract from all three cultivars demonstrates noteworthy results, surpassing the efficacy of other solvent extracts against the antioxidant activity.

Keywords Antioxidant, Barley, Cereals, Docking, *Hordeum vulgare* L

Background

Cereals have long been closely linked to food and drink, acting as a major natural energy source and providing numerous benefits for human health [1]. Barley is one of the oldest cereal crops that are grown. 10,500 years ago, the ancient Egyptians utilized it for the first time near the Nile River [2]. Barley can be classified according to its grain content into several categories, including normal, waxy (characterized by high-amylose starch content), high-glucan, and proanthocyanidin-free varieties [1]. Among the grains, barley has the highest quantities of β-glucan, followed by rye, wheat, and oats in decreasing order [3]. Oxidative stress, which is associated with the development of several diseases such as cancer, anemia, ischemia, diabetes, and cardiovascular diseases, is known to have a significant impact on the body's cellular processes [4]. Studies have demonstrated that barley grains contain phytochemical substances that have significant antioxidant qualities when evaluated in vitro [5]. The type of solvent employed during the extraction process has been discovered to affect the nature and quantity of secondary metabolites recovered from medicinal plants [6]. The structural dissimilarities of phenolic compounds influence their solubility in liquids of variable polarity. As a result, the solvent used for extraction and separation

*Correspondence:
Shahira Ezzat
shahira.ezzat@pharma.cu.edu.eg
[1] Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy, Future University in Egypt, Cairo 12311, Egypt
[2] Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr el Aini St., Cairo 11562, Egypt
[3] Department of Pharmacognosy, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza 12451, Egypt

processes can have a substantial impact on the yield of phytochemicals derived from plant sources [7]. The primary objective of this study was to examine the principal phytochemical constituents within the most potent barley plant extract. The extraction process encompassed the use of various solvents, including 70% ethanol, methanol, water, 80% methanol, and acetone. Additionally, the research evaluated the antioxidant capabilities of the barley extracts, and in silico investigations were conducted to assess the potential binding modes of various phytochemicals as ligands with polyphenol oxidases (PPOs) receptor proteins.

Antioxidant activity measurements

DPPH radical scavenging activity

The three varieties of barley with different solvents were evaluated for their antioxidant capacity using the DPPH as mentioned by Boly et al. [9]. Briefly, in a 96-well plate ($n=6$), 100 µL of freshly made DPPH reagent (0.1% in methanol) was together with 100 µL of the sample. The reaction was allowed to proceed for 30 min in the dark at room temperature. The subsequent decrease in DPPH color intensity was measured at 540 nm after the incubation period. FluoStar Omega, a microplate reader, was used to record the results. The following equation describes how data are expressed as means ± standard deviation, compared to torolox the standard drug.

$$\text{Percentage inhibition} = \frac{\text{Average absorbance of blank} - \text{Average absorbance of the test}}{\text{Average absorbance of blank}} \times 100$$

Methods

Plant materials collection and extraction

In August 2019, whole barley grains (*Hordeum vulgare* L.) from three distinct cultivars were gathered from the Agriculture Research Center in Egypt, sourced from different geographic locations. The three commonly cultivated *H. vulgare* varieties in Egypt, namely Giza 136 (G.136), Giza 127 (G.127), and Giza 131 (G.131), were utilized. Fifty grams of powdered plant material from each of the three cultivars underwent extraction using five distinct solvents: 100% methanol, 80% methanol in water, 70% ethanol in water, 80% acetone in water, and distilled water. The yield from cultivar G.136 was 5.9% with methanol, 6.12% with ethanol in water, 11.43% with 70% ethanol in water, 8.23% with 80% acetone in water, and 3.8% with distilled water. Meanwhile, for cultivar G.127, the yields were 4.42% with methanol, 7.23% with 80% methanol in water, 8.39%, 8.38% with 70% ethanol in water, 8.45% with 80% acetone in water, and 12.44% with distilled water. As for cultivar G.131, the yields were 3.19% with methanol, 8.52% with 80% methanol in water, 7.42% with 70% ethanol in water, 6.5% with 80% acetone in water, and 5.5% with distilled water. This process resulted in a total of 15 samples (three cultivars in five different solvents). To extract the compounds, all of the samples underwent sonication for 30 min, three times using 500 mL each time [8].

Ultra high-performance liquid chromatography-mass spectrometry analysis (UHLPC-MS)

The combined extracts were concentrated under reduced pressure. Ten milligrams of each extract was accurately weighed and then subjected to UHPLC-MS/MS analysis and measured their antioxidant activity.

Microsoft Excel® was used to analyze the data, and Graph Pad Prism 5® was used to get the IC_{50} value by converting the concentrations to their logarithmic value and choosing a nonlinear inhibitor regression equation. (log (inhibitor) vs. normalized response—variable slope equation) [10].

Ferric reducing antioxidant power assay (FRAP)

With a few minor adjustments to be performed in microplates, the Benzi et al. [11] method for the ferric reducing ability assay was used. In summary, a freshly made TPTZ reagent (300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM$FeCl_3$, respectively) was used. In a 96-well plate ($n=3$), 190 uL of freshly made TPTZ reagent was combined with 10 uL of the sample. The reaction was then allowed to sit at room temperature for 30 min while kept in the dark. The final measurement of the blue color after incubation was made at 593 nm. Data are displayed as means ± SD. FluoStar Omega, a microplate reader, was used to record the results. The ferric reducing ability of the samples is presented as µM TE/mg sample using the linear regression equation extracted from the calibration curve, compared to torolox the standard drug.

Molecular docking studies

A molecular docking study was conducted using the Discovery Studio 4.1 program and the LIPDOCKER methodology. The isolated component was docked against the active site of antioxidant, PDB (ID: 2Y9X). Heavy atoms were created, superfluous chains were eliminated, hydrogens were added, and the protein was purified. The

CHARMm forcefield and MMFF94 as a partial charge were used in the simulation. Fixed constraints and protein minimization were applied. The receptor binding site was located using the complicated ligand interaction site.

ADMET/TOPKAT prediction

The in silico ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) investigations were conducted using the Discovery Studio 4.1 program. These analyses aimed to predict the pharmacokinetic properties of the drug under examination, offering insights into its potential behavior within the body. The outcomes also yielded crucial structural information guiding the assessment of potential antioxidant activity. Graphical representations and numerical data were generated and presented. Additionally, the toxicity protocol TOPKAT was applied to the same set of compounds, evaluating various criteria including Ames Prediction, Carcinogenicity, and Rat Oral LD50 g/kg body weight.

Results

DPPH radical scavenging activity

In this experiment, the presence of hydrogen or electrons supplied by the antioxidant constituents in the samples resulted in the initial purple color of the DPPH radical changing to yellow. Figure 1 demonstrates that the antioxidant capacity of various sample extracts varies according to the polarity of the utilized solvents. As IC_{50} values decrease, the degree of antioxidative activity increases [12].

All tested varieties exhibited a significant difference with a P value less than 0.0001. However, variety G.131 methanol extract besides varieties G.127 and G.136 acetone extracts did not exhibit a significant difference with $P > 0.05$ in comparison with the standard drug, trolox, in terms of their antioxidant activity. This implies that they possess antioxidant properties. Besides, among all the varieties, variety G.136 acetone extract had the highest antioxidant activity (IC_{50}: 55.62 µg/ml), whereas variety G.127 acetone extract had the second-highest antioxidant activity (IC_{50}: 58.77 µg/ml). However, the methanolic extract of variety G.136 did not show any measurable DPPH activity.

Ferric reducing antioxidant power assay (FRAP)

A significant difference was found between the tested cultivars at a significance level of $P < 0.0001$, as illustrated by the antioxidant capacity of various sample extracts in Fig. 2. After comparing the acetone extracts of different varieties, the G.136 variety showed the highest antioxidant activity (447 µM trolox/mg extract), while the G.127 variety showed the subsequent highest antioxidant activity (426 µM trolox/mg extract). As expected from the results of DPPH, the ethanolic

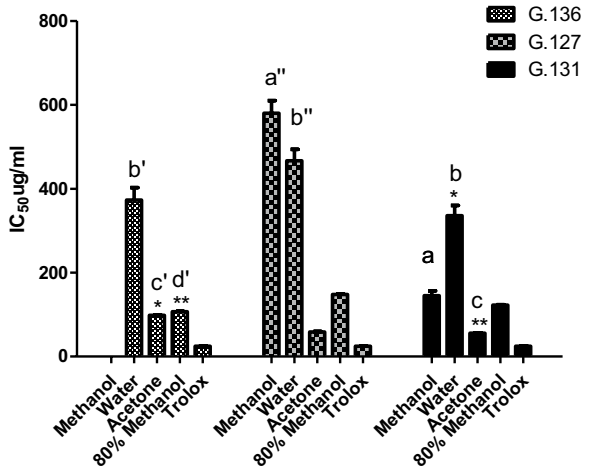

Fig. 1 DPPH activity of the three barley varieties in different solvents. Significant differences among means of different treatments were determined using Bonferroni posttests at $P < 0.001$ ($n = 3$) with all solvent extracts compared to each other. a, b, c, d significant difference compared to methanol, water, acetone, and 80% methanol of G.136, a′, b′, c′, d′ significant difference compared to methanol, water, acetone, and 80% methanol of G.127, a″, b″, c″, d″ significant difference compared to methanol, water, acetone, and 80% methanol of G.131, *corresponding to $P < 0.05$, **corresponding to $P < 0.01$, and the significance difference with $P < 0.001$

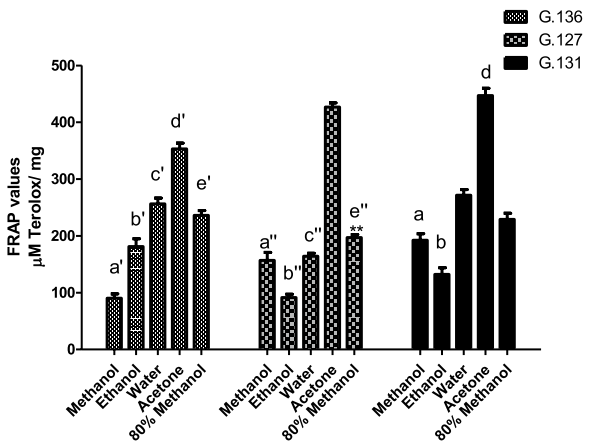

Fig. 2 FRAP activity of the three barley varieties in different solvents. Significant differences among means of different treatments were determined using Bonferroni posttests at $P < 0.001$ ($n = 3$) with all solvent extracts compared to each other. a, b, c, d, e significant difference compared to methanol, ethanol, water, acetone, and 80% methanol of G.136, a′, b′, c′, d′, e′ significant difference compared to methanol, ethanol, water, acetone, and 80% methanol of G.127, a″, b″, c″, d″, e″ significant difference compared to methanol, ethanol, water, acetone and 80% methanol of G.131, *corresponding to $P < 0.05$, **corresponding to $P < 0.01$, and the significance difference with $P < 0.001$

extracts of cultivars G.136 and G.127 showed the lowest levels of antioxidant activity (132.1 and 91.2 µM trolox/mg extract, respectively).

Ultra high-performance liquid chromatography-mass spectrometry analysis (UHLPC-MS)

Previously, sixty-four compounds using various solvents were discovered from all extracts (under publication). Because the acetone fraction has the highest activity in the previously mentioned antioxidant activity, we shed light on its prominent components. In the acetone fraction of the three cultivars, the major identified 18 compounds are shown in Table 1 and Fig. 3. Proanthocyanidin was the most prevalent chemical class among the identified phytochemicals in the three cultivars, followed by flavonoids and hordatines in the acetone fraction. Proanthocyanidin and flavonoid abundance were highest in G.131 of all the cultivars. However, cultivars, G.127, displayed the greatest quantity of hortatines (Fig. 4).

Based on a comparison of the detected component amounts in the three cultivars, quercetin 3-*O*-glucoside had the highest abundance among the three cultivars, with the G.131 cultivar exhibiting the highest concentration of the other varieties in addition to iso-orientin and hordatine B1; iso-orientin was also prominent in

Table 1 Metabolites using ultra high-performance liquid chromatography (UHPLC)-MS/MS of the acetone fraction in the three cultivars and their height

	RT	Compounds	Formula	Chemical class	M–H	Area of the compounds			Ref
						G.131	G.127	G.136	
1	0.238	Caffeic acid	$C_9H_8O_4$	Organic acids, phenolic compounds, and their derivatives	179.0334	0	0	4417	[13–15]
2	0.271	Guanine	$C_5H_5N_5O$	Amino acids	150.04236	12,033	23,042	17,730	[16]
3	0.486	Gluconic acid	$C_6H_{12}O_7$	Organic acids, phenolic compounds, and their derivatives	195.0506	195,682	133,080	170,648	[17]
4	0.865	Coumaroyle–OH–eagmatine	$C_{14}H_{20}N_4O_3$	Hordatines and hydroxycinnamic acid agmatines	291.1446	0	53,560	0	[18, 19]
5	1.406	Procyanadin B2	$C_{30}H_{26}O_{12}$	Proanthocyanidin, flavonoids, and their conjugates	577.1349	82,440	521,543	3,103,547	[20]
6	2.417	*p*-coumaric acid	$C_9H_8O_3$	Organic acids, phenolic compounds, and their derivatives	163.0391	0	7449	0	[13, 15, 21]
7	2.638	Adenin	$C_5H_5N_5$	Amino acids	134.04855	16,016	9048	0	[22]
8	3.327	Hordatine A1	$C_{28}H_{38}N_8O_5$	Hordatines and hydroxycinnamic acid agmatines	565.28967	17,631	0	0	[18]
9	3.736	Hordatine A glucoside	$C_{34}H_{48}O_9N_8$	Hordatines and hydroxycinnamic acid agmatines	711.34607	0	0	28,424	[18]
10	4.103	Tricin	$C_{17}H_{14}O_7$	Proanthocyanidin, flavonoids, and their conjugates	329.0659	510,883	556,177	7,078,250	[13, 15, 21]
11	4.124	Isovitexin 7-*O*-rhamnosyl-glucoside	$C_{33}H_{40}O_{19}$	Proanthocyanidin, Flavonoids and their conjugates	739.2091	47,112	41,444	0	[19]
12	4.768	Prodelphinidin B3	$C_{30}H_{26}O_{13}$	Proanthocyanidin, flavonoids, and their conjugates	593.13025	125,598	1,879,499	74,140	[21]
13	4.971	Hordatine B1	$C_{29}H_{40}N_8O_6$	Hordatines and hydroxycinnamic acid agmatines	595.2935	1,199,896	1,811,832	125,598	[18, 19]
14	5.731	Iso-orientin	$C_{21}H_{20}O_{11}$	Proanthocyanidin, flavonoids, and their conjugates	447.092375	6,005,607	5,952,463	386,493	[18, 23, 24] [19]
15	5.741	Quercetin 3-*O*-glucoside	$C_{21}H_{20}O_{11}$	Proanthocyanidin, flavonoids, and their conjugates	447.092375	40,335,493	13,767,985	4,707,879	[25]
16	5.756	Naringenin	$C_{15}H_{12}O_5$	Proanthocyanidin, flavonoids, and their conjugates	271.0604	268,109	0	0	[26, 27]
17	5.956	Canrenone	$C_{22}H_{28}O_3$	Steroids	339.20102	224,607	0	0	[28, 29]
18	6.937	Ferulic acid glucoside	$C_{16}H_{20}O_9$	Organic acids, phenolic compounds, and their derivatives	355.1031	107,234	0	0	[13, 19]

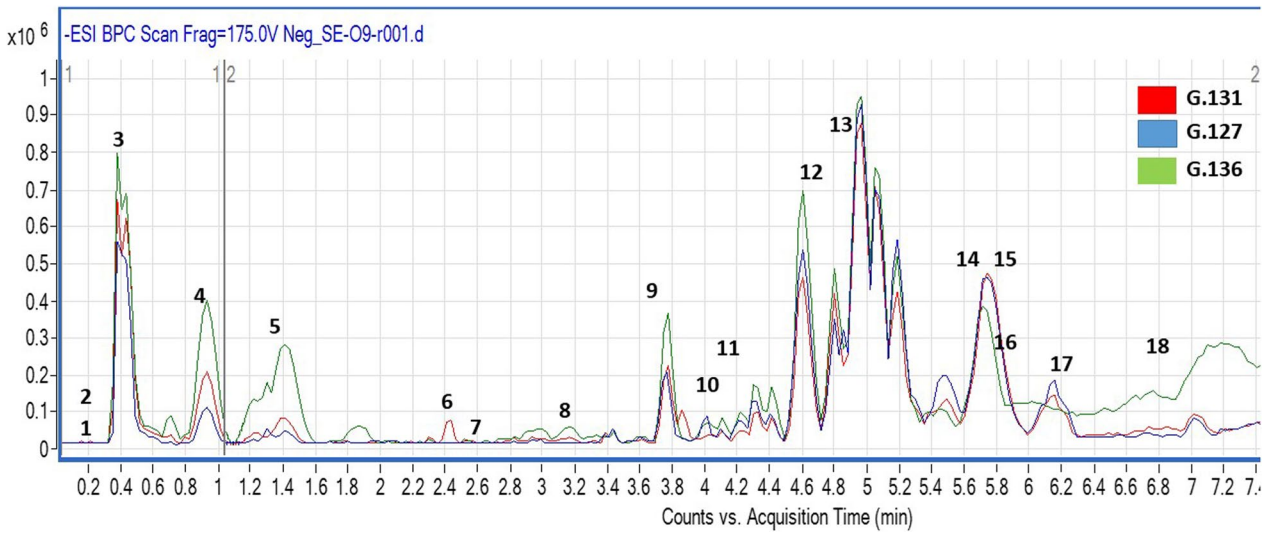

Fig. 3 Acetone fraction chromatogram of the three barley cultivars

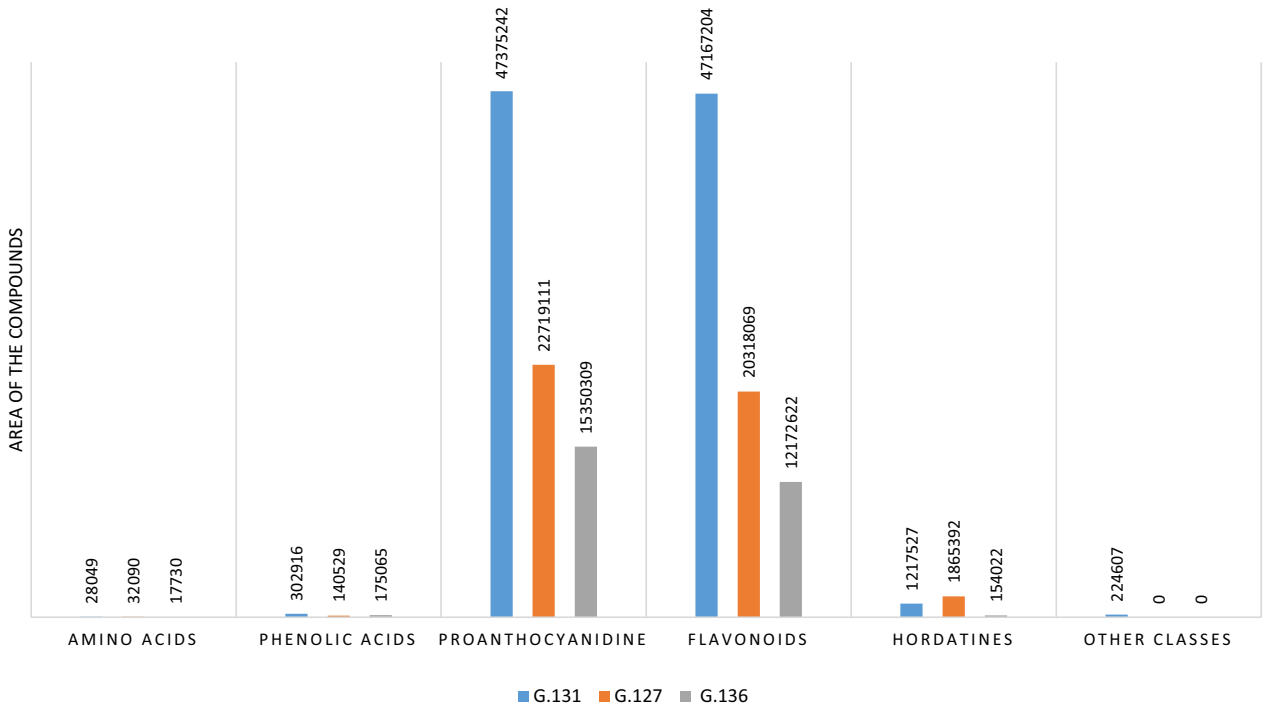

Fig. 4 Chemical classes among the three varieties

G.127 cultivar as well as prodelphinidin B3. Moreover, the main compounds in variety G.136 were tricin and procyanadin B2 as shown in Fig. 5.

Molecular docking studies

Molecular docking

The polyphenol oxidase enzyme PPO, which was obtained from the Protein Data Bank (PDB ID: 2Y9X), was docked to the identified phytochemicals and trolox, as reference antioxidant standard, to determine their potential binding mechanisms and virtual binding affinities. Docking of the 18 major detected compounds using the LIPDOCKER protocol after ligand preparation showed a LipDock score ranging from (−61.3546 to −143.402) (Table 2). Trolox showed hydrogen bond

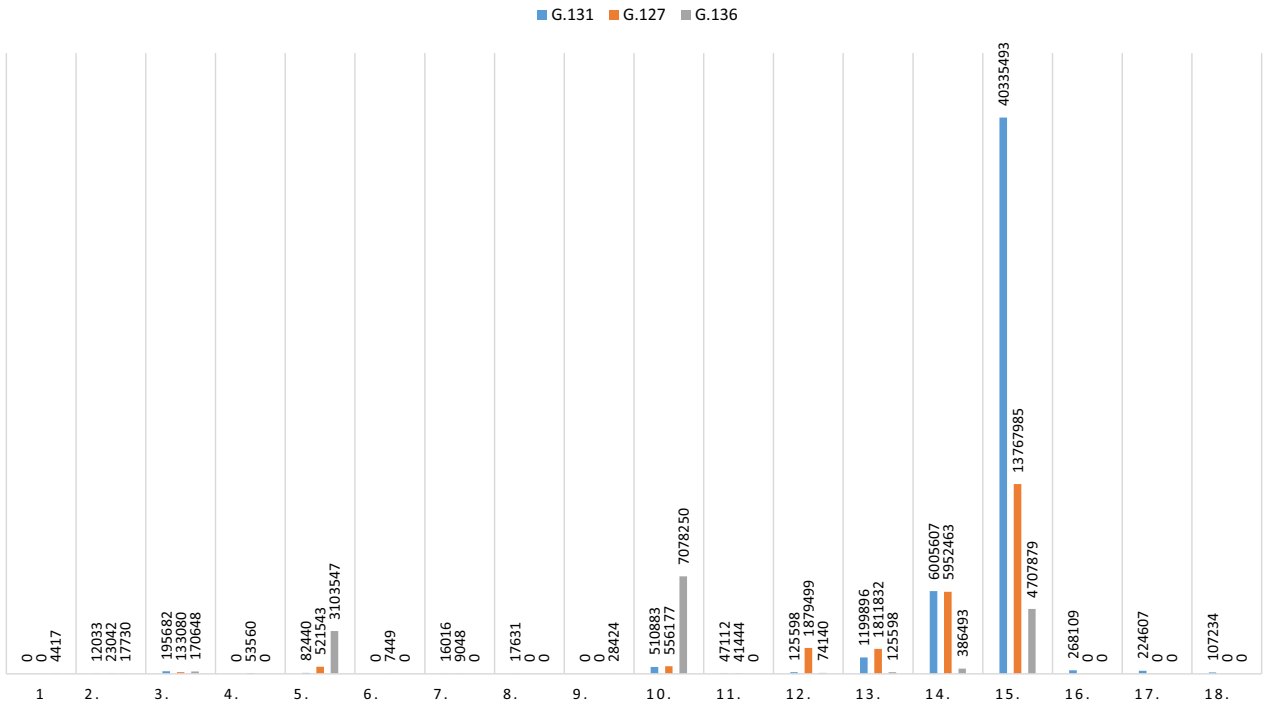

Fig. 5 Dominant compounds in the three barley cultivars (G.131, G.127 and G.136)

Table 2 LipDock score of the dominant compounds beside their amino acid interactions along with the reference trolox and the ligand drug

	Compounds	LipDock score	Key amino acid interaction	
			Hydrogen bond	Pi-bond
1	Caffeic acid	86.5240		Thr 84
2	Guanine	74.4313		Thr 84
3	Gluconic acid	93.0249	Trp136, Ile217, Ala221, Ile148, Trp138, Gly149	–
4	Coumaroyle–OH–eagmatine	117.4070	Ser282, Met280, Asn260, His259, Asn81	–
5	Procyanadin B2	132.7140	Glu322, Asn81, Ala246, Asn320, Tyr65	Val283, Ala246
6	*p*-coumaric acid	75.5750	–	Val283
7	Adenin	70.6453	His85	
8	Hordatine A1	143.4020	–, Arg321, His85, Thr84, Asn81, Asn320, Thr324, His244	Val283
9	Hordatine A glucoside	110.0050	His259, Asn260, Thr84, Thr324, Glu322	Ala246, Val247, Cys83
10	Tricin	103.7580	His85, Cys83, Ala323 Val283, His85, Ala80	–
11	Isovitexin 7-*O*-rhamnosylglucoside	124.8250	Tyr65, Tyr78	Ala323, Ala80, Pro284, Val283
12	Prodelphinidin B3	141.8140	Cys83, Asn81, Glu322 His244	Val283, Ala246,
13	Hordatine B1	138.9150	Asn81, His85, Arg321, Glu322, Ala323, Thr84	Val283
14	Iso-orientin	105.0620	Cys83, Asn81, Thr324, Ala323, Cys83, His85	Val283, His244,
15	Quercetin 3-*O*-glucoside	122.8930	Ala323, Tyr65, Tyr78	Ala80, Pro284, Val283, Ala323
16	Naringenin	108.7270	Arg321, Gly86,	His251, Ala246, Ala250, Val247, Arg321
17	Canrenone	98.0593	–	His85, His244, Cys83, Val283
18	Ferulic acid glucoside	115.5120	His85, Glu322	Val283, Ala323, Glu322
19	Trolox	80.2885	His85, Asn81, Glu322	His 244
20	2Y9X	61.4536	Asn243, Met319	Ala246, Val88, Thr87, Arg321

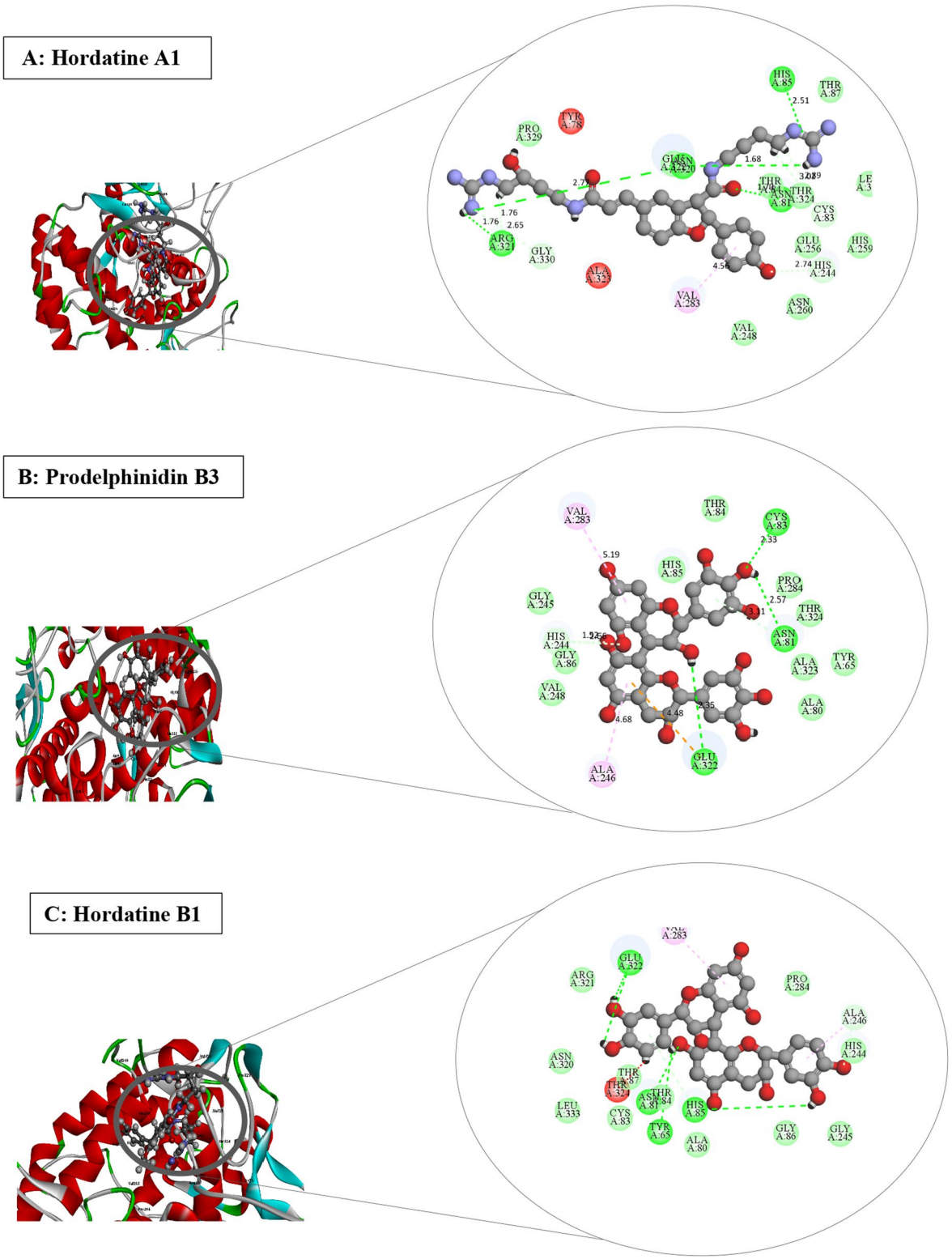

Fig. 6 2D binding mode of **A**: hordatine A1, **B**: prodelphinidin B3, **C**: hordatine B1, **D**: procyanadin B2, **E**: isovitexin 7-*O*-glucoside along with reference compound, **F**: trolox and the ligand drug, **G**: 2Y9X

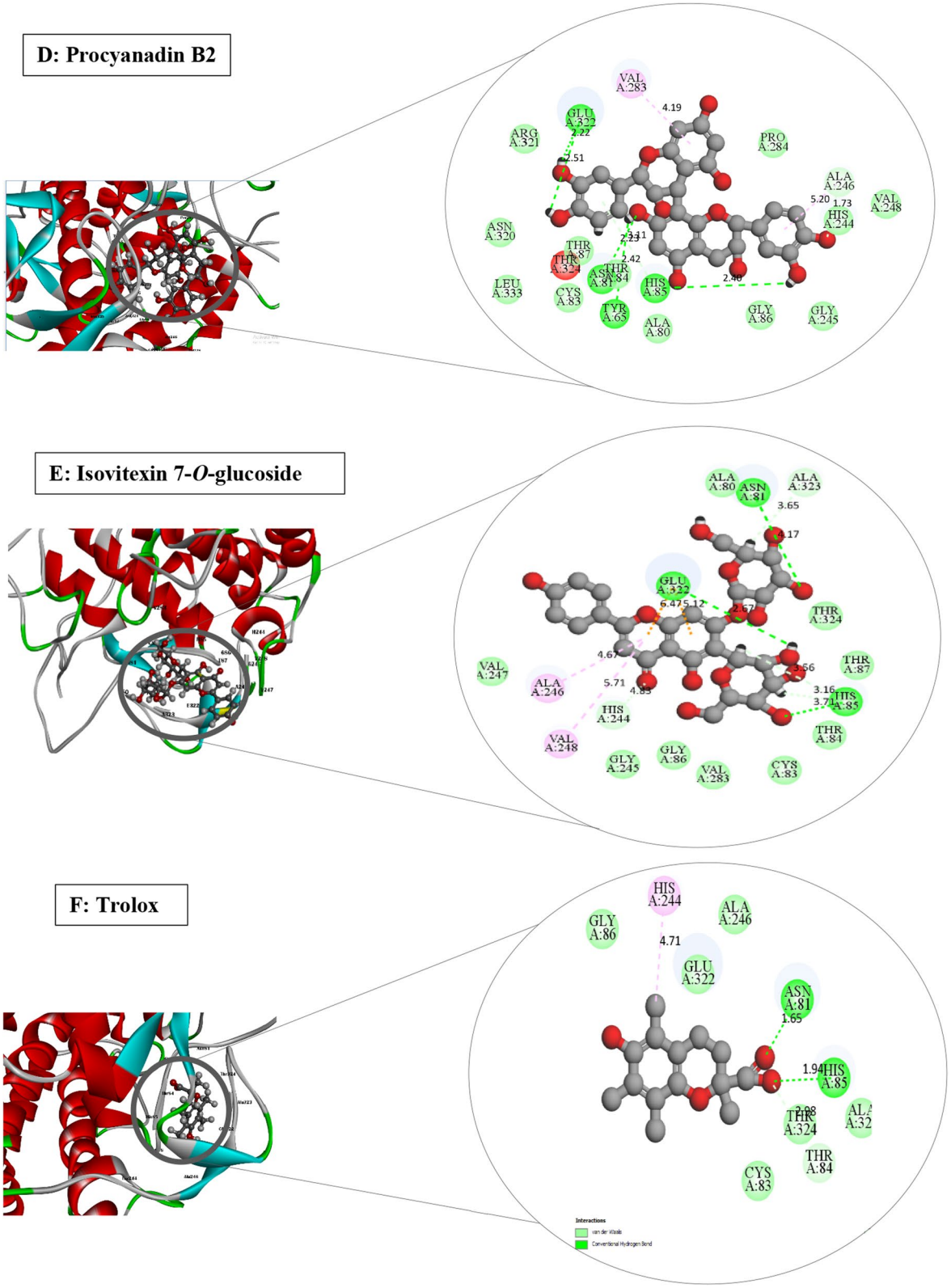

Fig. 6 continued

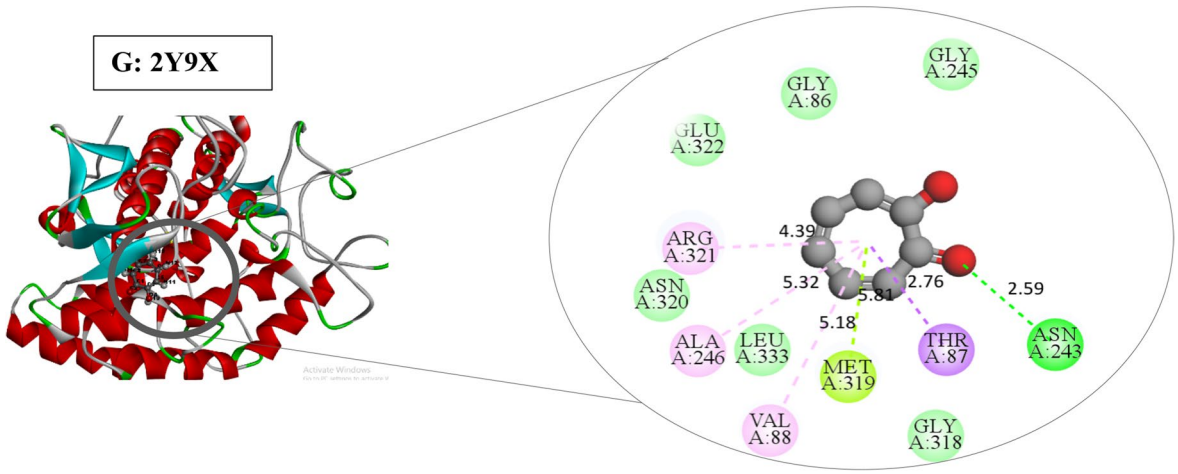

Fig. 6 continued

interaction with the essential amino acids (His85, Glu322, and Asn81) and hydrophobic interaction with (His 244).

Hordatine A1, prodelphinidin B3, hordatine B1, and procyanadin B2 showed the highest LipDock interaction energy score relative to trolox (Fig. 6), in addition to their highest abundance in the LCMS/MS results. Moreover, hordatine A1 and prodelphinidin B3 shared the same binding interaction with the essential amino acids as trolox (His85, Glu322, Asn81, and Val283) that showed a better stability along with the LipDock score.

In vitro predictive Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) study

The ADMET investigation, carried out using Discovery Studio 4.1 Software, focused on the molecular composition of the compound and included the computation of various parameters [30]. These parameters included: ADMET solubility level, Blood Brain Barrier Level (BBB LEV), and CYP2D6. Most of the compounds in the ADMET plot exhibited BBB levels ranging between 3 and 4. In the HIA plot, a significant portion of the compounds were located outside the 99% ellipse. Furthermore, many of these compounds had an ADME aqueous solubility rating falling between 3 and 4. The CYP2D6 score serves as an indicator of whether a specific chemical structure is inhibitory or non-inhibitory to the cytochrome P450 2D6 enzyme.

The key property, PSA (polar surface area), is a factor associated with drug bioavailability. Generally, molecules with a PSA greater than 240 are assumed to have limited bioavailability when passively absorbed (Fig. 7; Table 3).

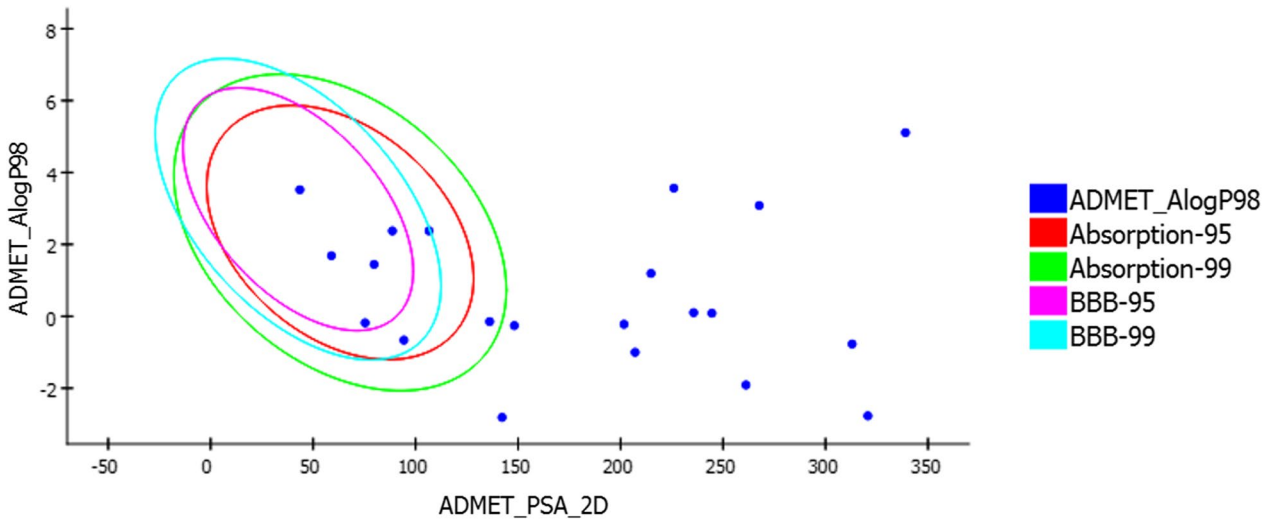

Fig. 7 ADMET Plot of the 2D polar surface area (PSA_2D) against calculated ALogP98 for examined compounds

Table 3 Computer-aided ADMET screening and TOPKAT Ames Toxicity study results of the identified compounds

	Compounds	ADMET solubility level	BBB level	CYP 2D6[f]	Hepatotoxic prediction	TOPKAT WOE prediction	TOPKAT Ames prediction	TOPKAT Rat Oral LD50 g/kg body weight
1	Caffeic acid	4	3	FALSE	FALSE	Non-carcinogen	Non-mutagen	1.63246
2	Guanine	4	3	FALSE	TRUE	Non-carcinogen	Non-mutagen	3.78993
3	Gluconic acid	5	4	FALSE	FALSE	Non-carcinogen	Non-mutagen	2.72903
4	Coumaroyle-OH-eagmatine	4	4	FALSE	TRUE	Non-carcinogen	Non-mutagen	0.864996
5	Procyanadin B2	1	4	TRUE	TRUE	Non-carcinogen	Non-mutagen	3.02939
6	*p*-coumaric acid	4	3	FALSE	FALSE	Carcinogen	Non-mutagen	1.35061
7	Adenin	4	3	FALSE	TRUE	Carcinogen	Mutagen	0.521014
8	Hordatine A1	3	4	FALSE	FALSE	Non-carcinogen	Non-mutagen	5.01923
9	Hordatine A glucoside	1	4	FALSE	FALSE	Non-carcinogen	Non-mutagen	19.2151
10	Tricin	3	3	FALSE	TRUE	Non-carcinogen	Non-mutagen	0.534205
11	Isovitexin 7-*O*-rhamnosylglucoside	0	4	FALSE	TRUE	Non-carcinogen	Non-mutagen	3.85073
12	Prodelphinidin B3	0	4	TRUE	TRUE	Non-carcinogen	Non-mutagen	2.53554
13	Hordatine B1	3	4	FALSE	FALSE	Non-carcinogen	Non-mutagen	7.97171
14	Iso-orientin	3	4	FALSE	TRUE	Non-carcinogen	Non-mutagen	1.32758
15	Quercetin 3-*O*-glucoside	3	4	FALSE	FALSE	Non-carcinogen	Non-mutagen	0.335631
16	Naringenin	3	3	TRUE	TRUE	Non-carcinogen	Non-mutagen	1.57835
17	Canrenone	2	1	FALSE	FALSE	Carcinogen	Non-mutagen	2.70998
18	Ferulic acid glucoside	4	4	FALSE	FALSE	Non-carcinogen	Non-mutagen	4.73868

TOPKAT toxicity studies

The compounds that were previously prepared underwent (TOPKAT) toxicity protocol [31], which involved evaluating them based on specific criteria, including Ames Prediction, Hepatotoxic Prediction, Rat Oral LD_{50}, and Carcinogenic Potency. This methodology was designed to gauge the potential toxicity of newly developed substances (Table 3).

Discussion

Natural antioxidants are significantly more beneficial and efficient in combating oxidative stress when compared to their synthetic counterparts. Medications derived from plant products are considered safer for consumption [32].

The DPPH radical dot assay is commonly employed to evaluate the free radical scavenging capabilities of an antioxidant molecule. It is recognized as a standard and straightforward colorimetric method for assessing antioxidant properties [33].

It is noteworthy that the acetone extract from variety G.136 exhibited the most substantial antioxidant activity. Following closely, the acetone extract derived from variety G.127 demonstrated the second-highest antioxidant potency. Conversely, it is essential to highlight that the methanolic extract obtained from variety G.131 did not manifest any detectable DPPH activity.

The FRAP assay stands out as a straightforward, rapid, and cost-effective direct technique for gauging the total antioxidant activity of reductive antioxidants present in a test sample [34].

In line with the findings from the DPPH assay, it was observed that the G.136 variety exhibited the highest antioxidant activity, followed by the G.127 variety with a slightly lower but still significant antioxidant activity. Interestingly, in contrast with the acetone extracts discussed earlier, the ethanolic extracts of cultivars G.136 and G.127 displayed the lowest levels of antioxidant activity.

Numerous studies have suggested that pure water is not an efficient solvent for extracting polyphenols due to their higher solubility in solvents that are less polar than water [35]. Comparable findings were published by Zhu et al. [36] concerning the significant antioxidant activity of the Chinese-grown barley acetone extract.

Among the identified phytochemical classes in the three cultivars, proanthocyanidins were the most prevalent, followed by flavonoids and hordatines in the acetone fraction. Specifically, proanthocyanidins and flavonoids

were most abundant in G.131, while G.127 exhibited the highest quantity of hordatines. Quercetin 3-*O*-glucoside was the most abundant component across all cultivars, with G.131 having the highest concentration of this compound compared to the other varieties.

A docking study was applied on the most prominent compounds in the acetone fractions, hordatine A1, prodelphinidin B3, hordatine B1, and procyanidin B2 exhibited the highest LipDock interaction energy scores compared to trolox.

Furthermore, these compounds also demonstrated the highest abundance in the LCMS/MS results. Likewise, these compounds previously revealed antioxidant activity [37, 38].

After making ADMET and TOPKAT studies of the dominant compounds in the acetone fractions, the compounds' Absorption, Distribution, Metabolism, and Excretion Toxicity could be determined.

This suggests that they are unlikely to permeate the blood–brain barrier and, consequently, are unlikely to cause adverse effects in the central nervous system (CNS) adverse effects. They are likely to have limited absorption in the intestines suggesting that they possess good aqueous solubility; the expected values for these compounds indicate good passive oral absorption for most of them, which is a positive attribute for their pharmacological effectiveness.

In this context, since these compounds are classified as non-inhibitors of CYP2D6, it suggests that their usage is not likely to lead to adverse effects such as liver impairment.

The results of all TOPKAT Ames probabilities, applications, and scores indicated that these compounds are non-mutagenic and non-carcinogenic, and they fell within the anticipated ranges.

Conclusion

The present study offers valuable insights into the antioxidant applications of different solvents extracts of three different cultivars. The acetone extracts of the three cultivars showed the best results compared to other solvents. G.136 variety showed the highest level of antioxidant activity in both the DPPH and FRAP assays. A docking study was conducted for the 18 major compounds in the acetone fractions of the three varieties followed by ADMET and TOPKAT studies. Hordatine A1, prodelphinidin B3, hordatine B1, procyanidin B2, and isovitexin 7-*O*-glucoside demonstrated the highest LipDock scores when compared to the reference standard drug. Additionally, these compounds exhibited the highest areas among other constituents in the three cultivars. The results of the current study could offer valuable insights for defining new research avenues regarding the utilization and applications of the examined extracts with specific solvents as pharmaceutical and nutraceutical agents.

Abbreviations

DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power assay
ADMET	Absorption, Distribution, Metabolism, Excretion, and Toxicity
TOPKAT	Toxicity Prediction by Komputer Assisted Technology
PPOs	Polyphenol oxidases
PDB	Protein Data Bank
(UHPLC)-MS/MS	Ultra high-performance liquid chromatography
PSA	Polar surface area

Acknowledgements

Not applicable.

Author contributions

Conceptualization was performed by O.E.; S.E.; and W.E.; data curation by O.E.; investigation by O.E.; methodology by O.E.; supervision by S.E.; W.E.; A.E.; and E.A.; visualization by O.E.; S.E.; and W.E.; writing—original draft by O.E. and W.E.; writing—review & editing by O.E.; S.E.; W.E.; and E.A. All authors read and approved the final manuscript.

Funding

This research received no external funding

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the research ethics committee, Faculty of Pharmacy, Cairo University, serial number MP (2413).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Plant authentication

The Whole barley grains (*Hordeum vulgare* L.) from three distinct cultivars were gathered from the AgricultureResearch Center in Egypt and sourced from different geographic locations in Egypt. The three commonly cultivated*H. vulgare* varieties in Egypt, namely Giza 136 (G.136), Giza 127 (G.127), and Giza 131 (G.131) were utilized.

Received: 11 February 2024 Accepted: 9 May 2024
Published online: 17 May 2024

References

1. Gangopadhyay N et al (2015) A review of extraction and analysis of bioactives in oat and barley and scope for use of novel food processing technologies. Molecules 20(6):10884–10909
2. Emilia-Ancuța B et al (2019) Barley (*Hordeum vulgare* l.): medicinal and therapeutic uses—review. Hop Med. Plants
3. Sakellariou M, Mylona PV (2020) New uses for traditional crops: the case of barley biofortification. Agronomy 10(12):1964
4. Ravipati AS et al (2012) Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content. BMC Complement Altern Med 12(1):1–14
5. Abbasi AM et al (2009) Medicinal plants used for the treatment of jaundice and hepatitis based on socio-economic documentation. Afr J Biotechnol 8(8):66

6. Dirar A et al (2019) Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. S Afr J Bot 120:261–267
7. Złotek U et al (2016) The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. Saudi J Biol Sci 23(5):628–633
8. Salem MA et al (2020) Optimization of an extraction solvent for angiotensin-converting enzyme inhibitors from *Hibiscus sabdariffa* L. based on its UPLC-MS/MS metabolic profiling. Molecules 25(10):2307
9. Boly R et al (2016) DPPH free radical scavenging activity of two extracts from *Agelanthus dodoneifolius* (Loranthaceae) leaves. Int J Toxicol Pharmacol Res 8(1):29–34
10. Chen Z, Bertin R, Froldi G (2013) EC50 estimation of antioxidant activity in DPPH assay using several statistical programs. Food Chem 138(1):414–420
11. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239(1):70–76
12. Chen Y et al (2019) Comparative study on the phytochemical profiles and cellular antioxidant activity of phenolics extracted from barley malts processed under different roasting temperatures. Food Funct 10(4):2176–2185
13. Verardo V et al (2008) Distribution of bound hydroxycinnamic acids and their glycosyl esters in Barley (*Hordeum vulgare* L.) air-classified flour: comparative study between reversed phase-high performance chromatography-mass spectrometry (RP-HPLC/MS) and spectrophotometric analysis. J Agric Food Chem 56(24):11900–11905
14. Sawada Y et al (2009) Widely targeted metabolomics based on large-scale MS/MS data for elucidating metabolite accumulation patterns in plants. Plant Cell Physiol 50(1):37–47
15. Sinosaki N et al (2020) Structural study of phenolic acids by triple quadrupole mass spectrometry with electrospray ionization in negative mode and H/D isotopic exchange. J Braz Chem Soc 31:402–408
16. Jones DJ et al (2022) Determination of N7-glycidamide guanine adducts in human blood DNA following exposure to dietary acrylamide using liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 36(6):e9245
17. Felipe DF et al (2014) Phytochemical analysis of *Pfaffia glomerata* inflorescences by LC-ESI-MS/MS. Molecules 19(10):15720–15734
18. Pihlava J-M (2014) Identification of hordatines and other phenolamides in barley (*Hordeum vulgare*) and beer by UPLC-QTOF-MS. J Cereal Sci 60(3):645–652
19. Piasecka A et al (2015) Combined mass spectrometric and chromatographic methods for in-depth analysis of phenolic secondary metabolites in barley leaves. J Mass Spectrom 50(3):513–532
20. Poupard P et al (2011) Characterization of procyanidin B2 oxidation products in an apple juice model solution and confirmation of their presence in apple juice by high-performance liquid chromatography coupled to electrospray ion trap mass spectrometry. J Mass Spectrom 46(11):1186–1197
21. Koistinen VM, Hanhineva K (2017) Mass spectrometry-based analysis of whole-grain phytochemicals. Crit Rev Food Sci Nutr 57(8):1688–1709
22. Shi J et al (2011) Development of a robust and sensitive LC–MS/MS method for the determination of adenine in plasma of different species and its application to in vivo studies. J Pharm Biomed Anal 56(4):778–784
23. Lee JH et al (2016) Elucidation of phenolic antioxidants in barley seedlings (*Hordeum vulgare* L.) by UPLC-PDA-ESI/MS and screening for their contents at different harvest times. J Funct Foods 26:667–680
24. Wu L-Z et al (2013) Characterization of aromatic glycosides in the extracts of Trollius species by ultra high-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. J Pharm Biomed Anal 75:55–63
25. Keskes H et al (2017) LC–MS–MS and GC–MS analyses of biologically active extracts and fractions from Tunisian Juniperus phoenice leaves. Pharm Biol 55(1):88–95
26. Hajji T et al (2018) Identification and characterization of phenolic compounds extracted from barley husks by LC-MS and antioxidant activity in vitro. J Cereal Sci 81:83–90
27. Katsumata S et al (2017) Identification of sternbin and naringenin as detoxified metabolites from the rice flavanone phytoalexin sakuranetin by *Pyricularia oryzae*. Chem Biodivers 14(2):e1600240
28. Ferreira-Nunes R et al (2023) Liquid chromatography-mass spectrometry for simultaneous determination of spironolactone and canrenone in plasma samples. Braz J Pharm Sci 59:e21626
29. Guo P et al (2020) Simultaneous determination of canrenone, digoxin and tolvaptan by UHPLC–MS/MS: application in heart failure patients. Bioanalysis 12(9):569–582
30. Sabry MA et al (2022) New thiazole-based derivatives as EGFR/HER2 and DHFR inhibitors: synthesis, molecular modeling simulations and anticancer activity. Eur J Med Chem 241:114661
31. Ajala A et al (2023) QSAR, simulation techniques, and ADMET/pharmacokinetics assessment of a set of compounds that target MAO-B as anti-Alzheimer agent. Future J Pharm Sci 9(1):4
32. Saleem M et al (2023) Investigation of antioxidant and antibacterial effects of citrus fruits peels extracts using different extracting agents: phytochemical analysis with in silico studies. Heliyon 9(4):66
33. Mishra K, Ojha H, Chaudhury NK (2012) Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results. Food Chem 130(4):1036–1043
34. Benzie IF, Devaki M (2018) The ferric reducing/antioxidant power (FRAP) assay for non-enzymatic antioxidant capacity: concepts, procedures, limitations and applications. Meas Antioxid Activity Capacity Recent Trends Appl 66:77–106
35. Sepahpour S et al (2018) Comparative analysis of chemical composition, antioxidant activity and quantitative characterization of some phenolic compounds in selected herbs and spices in different solvent extraction systems. Molecules 23(2):402
36. Zhu Y et al (2015) Phenolics content, antioxidant and antiproliferative activities of dehulled highland barley (*Hordeum vulgare* L.). J Funct Foods 19:439–450
37. Becker D et al (2022) Isolation and characterisation of hordatine-rich fractions from Brewer's spent grain and their biological activity on α-glucosidase and glycogen phosphorylase α. Sustainability 14(14):8421
38. Tang Y et al (2021) Impact of germination pretreatment on the polyphenol profile, antioxidant activities, and physicochemical properties of three color cultivars of highland barley. J Cereal Sci 97:103152

Publisher's Note