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Original article

Studies of phytochemical constituents by UPLC-QTOF-MS/MS of black hulless barley bran and its antioxidation and α-glucosidase inhibition effect

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ABSTRACT

Anthocyanidins and flavonoids are important components in plant. In present study, the anthocyanidins and flavonoids in black hulless barley bran were identified by operating UPLC-QTOF-MS/MS. Among them, chrysoeriol 7-O-glucouronid, luteolin 7-*O*-glucouronid, chrysoeriol, and luteolin were quantified by using UPLC. Additionally, the total phenolic content (TPC), total flavonoids content (TFC) and total anthocyanin content (TAC) were determined. Furthermore, the antioxidant activity (via ferric reducing, DPPH and ABTS method) and α-glucosidase inhibition effect were estimated. From the results, fifty-one constituents comprised 19 anthocyanidins and 32 flavonoids were detected in hulless barley bran fractions. Wherein compounds **1** and **9**–**19** (belong to anthocyanidins), and **21**, **25**–**32**, **35**–**42**, **44**–**50** (belong to flavonoids) were first identified in hulless barley. The contents of chrysoeriol 7-O-glucouronid luteolin 7-*O*-glucouronid, chrysoeriol and luteolin were higher in E80% than that of in E40%, but were not detected in E20%. Another, the TAC exhibited the trend of E40% > E80% > E20%, and the TPC and TFC showed same trend of E40% > E20% > E80%. The higher TPC and TFC showed stronger antioxidant activity. However, no significant correlation between TAC, TPC, TFC and α-glucosidase inhibitory activity was observed. These results provided the anthocyanidins and flavonoids information, antioxidant value and α-glucosidase inhibition potential, which could provide reference in the development of black hulless barley bran.

1. Introduction

Hulless barley, *Hordeum vulgare* L. *var. Nudum* Hook.f., is a widely cultivated cereal crop mainly growing in highland areas around the world, including Qinghai-Tibet Plateau in China, central Siberian Highlands in Russia, the north-western Hinalayas, and highland areas of Canada and Germany (Zhang et al., 2019). Hulless barley is a special cereal crop which possess high content of protein, vitamins and dietary fiber,but low content of sugar and fat, meeting the needs of a modern and healthy lifestyle (Guo et al., 2020). In addition, β-glucan content in huless barley is rich, which have the potential to lower the synthesis of cholesterol and reduce the concentration of low-density blood lipid (Zhang et al., 2019). Moreover, abundant phenolic compounds in hulless barley, including flavonoids, phenolic acids, and anthocyanins (Ge et al., 2020;Yang et al., 2023), endowed it with the ability of anticancer, antimicrobial, antioxidative properties, and reduction of the risk of chronic diseases (Idehen et al., 2017; Hu et al., 2023; Olatunji et al., 2022; Jayeoye et al., 2021). So hulless barley has attracted increased focus because of its beneficial composition and health value in recent years.

Whole grains consist of starchy endosperm, germ, and bran (Liu, 2007). The hull and bran fractions of cereals was usually discarded in order to produce various types of products for consumption. However, the discarded fractions of hulless barley, composed of the bran and hull, accounting for 30 % of the total grain weight, are waste and poorly processed (Jadhav et al., 1998). The hulless barley bran contain

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amounts of phytochemical components, such as β-glucans, dietary fibre, flavonoids, phenolic acids, and anthocyanin, which act as functional phytochemicals in hull-less barley grain (Šimić et al., 2019; Xiang et al., 2021). In two hull-less and two waxy barleys, the β-glucan content in bran fractions was of the highest (Wiege et al., 2016). While the concentration of β-glucan in shorts fraction was highest (8.12–13.01 %), compared with bran and flour in six hull-less barley cultivars (Zheng et al., (2011). Besides, the bran of hulless barley expressed 68 % and 56 % of the total ABTS antioxidant capacity and the total DPPH antioxidant capacity,respectively, despite it accounted for 7 % of the total grain weight (Xiang et al., 2021). Furthermore, ferulic acid in bran was also highest in hull-less barley (Šimić et al., 2019), similar result showing ferulic acid content was highest in the outer layers in cereals (López-Pereaa et al., 2019; Ndolo et al., 2013; Vaher et al., 2010).

Hulless barley are richer in anthocyanins and flavonoids, but little report exists on the bran of black grains. Thus, in present study, the anthocyanins and flavonoids in black hulless barley bran were identified by using ultra-high-performance liquid chromatography-diode array with quadrupole time-of-flight and tandem mass spectrometry (UPLC-QTOF-MS/MS). Besides, the four flavonoids was quantified by operating ultra high performance liquid chromatography (UPLC). Furthermore, the total phenolic content (TPC), total flavonoid content (TFC) and the total anthocyanin content (TAC) in black hulless barley bran were determined. The antioxidant activities as well as α-glucosidase inhibition effect were also evaluated. This study can provide valuable reference for the utilization of black hulless barley, particularly for the consumption of anthocyanins and flavonoids of black barley bran.

2. Materials and methods

2.1. Materials

The ‘Longzi’, purchased from Tibet Chunguang Food Co. Ltd. (Xizang, China), is a hulless barley variety with black grain color, 10.1 kg bran was obtained from 100 kg hulless barley grain which was miled using a NZJ15/15 grain mill (Changzhou Weihai Machinery Technology Co., Ltd. Changzhou, China). Then these bran were ground into powder and screened through a 60-mesh sieve. These bran powder were kept at −20 °C for further tests.

2.2. Chemicals and reagents

Analytical-grade methanol were obtained from Chengdu Kelong Chemical Reagent Works (Chengdu, China). Chromatographic-grade methanol and acetonitrile were obtained from Sigma-Aldrich (St. Louis, MO, USA).

AB-8 Macroporous Resin was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Briefly, the resins were washed 3 times with distilled water, then soaked for 24 h with ethanol. Later the resins were washed thoroughly until no alcohol smell with distilled water, then soaked with 4 % HCl for 2 h. Again, the resins were washed to neutral with distilled water, then were soaked with 4 % NaOH for 2 h. Finally, the resins were kept washing until the washing fluid became neutral with distilled water. The water in the resins were removed by filtering before use.

2.3. Sample preparation

100 g sample bran was ultrasound extracted by 800 mL of petroleum ether at 40 °C for 1 h in the KH2200DE ultrasonic instrument (Kunshan Ultrasound Instrument Co., Jiangsu, China). The mixture was filtered. Then the filter residue was collected and dried in the shade. 600 mL of 80 % ethanol together with 0.1 % hydrochloric acid was added into the filter, and was extracted 3 times at 40 °C, each time for 40 min. The three filtrates were mixed and evaporated to 100 mL at 50 °C under vacuum with a rotary evaporator (Hei-VAP, Heidolph Instruments, Schwabach, Germany). Then 100 mL of sample solution was allowed to flow at a rate of 1.0 BV/h through the glass column (diameter 3 cm × 35 cm high), then was adsorbed for 1 h. Then the column was washed with 1000 mL of 0.5 % hydrochloric acid water at a flow rate 2 BV/h, and later washed with 1000 mL eluted solution (20 %, 40 %, 80 % ethanol solution containing 0.5 % hydrochloric acid, respectively), collected 20 %, 40 %, 80 % eluted part. The eluted solution was concentrated under reduced pressure at 50 °C and freeze-dried at −80 °C by FDU-2110 Freeze dryer (Tokyo Rikakikai CO., LTD., Tokyo, Japan). The eluted sample was obtained as follows: 20 % ethanol solution (E20%, 0.5557 g), 40 % ethanol solution (E40%, 0.8994 g), 80 % ethanol solution (E80%, 0.3341 g).

2.4. Anthocyanin and flavonoid compounds identification using UPLC-QTOF-MS/MS

To analyze anthocyanin and flavonoid compounds in the hulless barley bran, an ultra-high-performance liquid chromatography (UPLC) system (Waters Corporation; Milford, MA, USA) with a diode array detector (DAD) was performed. The detector was coupled to a Waters Xevo G2-XS quadrupole time-of-flight (Q-TOF) micro-mass spectrometer (Waters, Manchester, UK), equipped with an electrospray ionization (ESI) source acting on positive mode. Briefly, the chromatographic separation was operated by using the Waters BEH C18 column (2.1 mm × 100 mm, 1.7 μm particle size; Waters Corporation; Milford, MA, USA) as follows: the injection volume of 1 μL, as well as the elution was fulfilled in 18 min with a flow rate of 0.3 mL/min. The compositions of mobile phase were 0.1 % formic acid in water (solvents A) and acetonitrile (solvents B). The gradient conditions were settled as follows: 5 %–10 % B for 0–2 min, 10 %–20 % B for 2–10 min, 20 %–40 % B for 10–15 min, 40 %-70 % B for 15–17 min, 70 %–100 % B for 17–18 min. The DAD spectra for phenolic compounds were all examined at 320 nm. The mass spectrometer (MS) parameters were settled: capillary voltage of 2.5 kV, source default voltage of 80 V, source temperature at 250 °C, desolvation temperature at 120 °C, cone voltage of 40 V, cone gas flow of 50 L/h, as well as desolvation gas flow of 600 L/h. Finally, the MS analysis was accomplished by operating mass scanning from m/z 50 to 1500.

2.5. Quantification of flavonoid compounds using UPLC

The characteristic flavonoid compounds in extracts were determined according to the method of Xiang et al. (2021). An UPLC instrument, equipped with a DAD detector (Agilent LC1290 series, Agilent Technologies, USA), and a 100 mm × 2.1 mm, 1.7 μm particle size Waters BEH C18 (Waters Technologies, USA) were employed in analysis process. The gradient program of mobile phase was assigned as follows: 0–2 min for 5 %-10 % B, 2–10 min for 10 %-20 % B, 10–15 min for 20–40 % B, 15–17 min for 40 %-70 % B, 17–18 min for 70 %-95 % B, 18–20 min for 95 % B at the 0.3 mL/min flow rate. Then detection was carried out at 30 ℃ for column temperature with 1 μL injection volume and 350 nm detection wavelength. Compounds of four flavonoid were quantified with the corresponding standard compounds, and the contents were expressed as μg flavonoid/g dry weight (DW).

2.6. Total anthocyanin content determination

The total anthocyanin content (TAC) was evaluated by the method of Xia et al. (2022) with some modifications, by the Agilent LC-1260 high-performance liquid chromatography (HPLC) system (Agilent, Santa Clara, CA, USA) fitted with a diode array detector (DAD). Then chromatographic separation was performed with an Infinity Lab Poroshell 120 PFP column (4.6 × 100 mm, 2.7 μm particle size, Agilent, Santa Clara, CA, USA). Based on a previous study (Xia et al., 2021), the gradient program of mobile phase was set at 5 %–10 % B (0–10 min), 10 %–20 % B (10–20 min), 20 %–40 % B (20–35 min), 40 %–70 % B (35–40 min), 70 %–95 % B (40–45 min), with a flow rate of 0.8 mL/min. The

column temperature was the same as that in section 2.5 with injection volume of 5 μL and the detection wavelength of 525 nm. To evaluate TAC, the.Cyanidin-3-glucoside was utilized as an external standard. The regression equation was $y = 3.4613x - 13.562$, $R^2 = 0.9999$, with a linear range of 7.81 to 1000 μg/mL. The content of TAC were expressed as mg cyanidin-3-glucoside/g dry weight (DW).

2.7. Total phenolic content analysis

The total phenolic content (TPC) was tested according to the Folin-Ciocalteu colorimetric method. 20 μL Folin-Ciocateu reagent was mixed with 20 μL sample solution, shaken and kept at room temperature for 5 min. Then the mixture was added with 160 μL Na_2CO_3 solution (5 %, w/v, in water) and reacted at room temperature for 1 h, avoiding light. The absorbance of mixture was monitored at 765 nm. The regression equation of gallic acid was $y = 0.0069x + 0.0273$ ($R^2 = 0.9981$), with a linear range of 3.31 to 212 μg/mL. The content of TPC was expressed as milligram gallic acid equivalent (GAE) per gram.

2.8. Measurement of total flavonoid content

The total flavonoid content (TFC) was quantified using the method of Xiang et al. (2022), with some modifications. 20 μL sample solution was added with 10 μL 25 % (m/v) $NaNO_2$, mixed and maintained at room temperature for 6 min. Then 10 μL 10 % $AlCl_3$ was mixed and reacted for 5 min. Subsequently, 30 μL 1 mol/L NaOH together with 100 μL distilled water were added to the mixture. The the absorbance was determined at 510 nm. The concentration of TFC was calculated using a standard curve ($y = 0.0013x + 0.0037$, $R^2 = 0.9985$).

2.9. Analysis of antioxidant activity

The DPPH radical scavenging activity was measured by the method of Yu et al. (2021), with some modification. Briefly, 100 μL sample solution,diluted to the appropriate ratio, was mixted with 100 μL 128.5 μg/mL DPPH solution (dissolved in 80 % methanol), then was incubated at room temperature for 30 min in a darkness before determination at 517 nm. takeing Trolox as a positive control. Then the scavenging activity of DPPH radical was calculated as following.

$$Scavengingactivity(\%) = (1 - Asample/Acontrol) \times 100 \quad (1)$$

Where *A*sample was the absorbance of tested sample solutions, while *A*control was the absorbance of the control reaction where the sample was replaced by 80 % methanol.

The ABTS radical scavenging activity was assayed by the method of Yu et al. (2021), with modifications. The working ABTS reagent containing 2.45 mM $K_2S_2O_8$ and 7 mM ABTS, was incubated for 16 h without light. When the absorbance of ABTS reagent was 7.0, it was diluted by pH = 7.4 PBS solution.Later, the volume of 40 μL sample solution (diluted to the appropriate ratio) was incubated with ABTS reagent (160 μL) for 6 min at room temperature in a darkness. The absorbance was subsequently recorded at 734 nm. Trolox was also used as a positive control. The scavenging activity of ABTS radical was measured by the Eq.1.

The reducing power was determined as method described previously with some modifications (Dudonné et al., (2009). To prepare the working FRAP reagent, 0.3 M CH_3COONa (pH = 3.6), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ, in HCl), as well as 20 mM $FeCl_3$ was mixed in a ratio of 10:1:1. Then 30 μL sample solutions, which was diluted to the appropriate ratio, was incubated with 265 μL FRAP reagent at 37 °C in a water bath for 30 min. Similarly using Trolox as a positive control, the absorbance of 593 nm was measured. A sample blank test was also performed using sodium acetate buffer. The reduction activity of the extract was reflected by the solution absorbance. The sample owned stronger reducing power as with the bigger solution absorbance.

2.10. The inhibition effect of α-glucosidase

The α-glucosidase inhibition effect was measured according to the method described by Yang et al. (2021) with slight modifications. PBS (0.1 M, pH = 6.9) was used to prepare the reagent of α-glucosidase and pNPG. Sample solution (50 μL), which was diluted to the appropriate ratio, was incubated with α-glucosidase reagent (1U/mL, 500 μL) in a 37 ℃ water bath for 10 min, and then added with 50 μL of 5 mM pNPG. After kept in a 37 ℃ water bath for 5 min, 50 μL of 1 M Na_2CO_3 was added into the mixture system. Then the absorbance of 405 nm was recorded, by setting the absorbance as a positive control. To calculate the enzyme inhibition activity, the following equations were used:

$$Inhibitioneffect/\% = [1 - (A0 - A1)/(A2 - A3)] \times 100\% \quad (2)$$

A_0:sample + α-glucosidase + pNPG + Na_2CO_3
A_1:sample + PBS + pNPG + Na_2CO_3.
A_2:PBS + α-glucosidase + pNPG + Na_2CO_3.
A_3:PBS + PBS + pNPG + Na_2CO_3.

2.11. Statistical analysis

All determined experiments were performed in triplicate otherwise specified. The results were expressed as mean ± standard deviation (SD). The stastical analysis was determined by one-way ANOVA, followed by Student-Newman-Keuls (SNK) test (version 20.0, IBM SPSS Statistics, Armonk, NY USA). Pearson's correlation coefficient was used to determine the correlation analysis among TAC, TPC, TFC, four quantified flavonoids, antioxidant activity and inhibition of a-glucosidase. Value with $p < 0.05$ were consideried statistically significant.

3. Results and discussion

3.1. Identification of anthocyanin and flavonoid compounds

51 compounds including 19 anthocyanidins and 32 flavonoids in black hulless barley bran fractions were characterized and identified using authentic standards, published references, as well as MS/MS fragmentation patterns in total (Table 1). And the chemical structure, characteristics of secondary ion mass spectrum in positive, and fragmentation patterns of Compounds **26** and **38** were exhibited in Fig. 1. The MS spectra of the identified compounds in E80% fraction was supported in **Supplementary material 1**. The anthocyanidins contained 18 cyanidin derivatives and one pelargonidin 3-*O*-glucoside. The dominant flavonoids in extracts were chrysoeriol and luteolin, and the sugars were glucose, galactoside, glucuronide. From the Table 1, the extracts contain 0, 18 and 9 cyanidin derivatives for E20%, E40%, E80%, respectively. These results were consistent with TAC content determined in present study. Another, the extracts contain 0, 24 and 27 flavonoids for E20%, E40%, E80%, respectively.

Compound **1** displayed a molecular ion at *m/z* 733, together with a fragment ion at *m/z* 287 ($[M + H\text{-}162\text{–}162\text{–}162]^+$ loss of three glucose moieties, established as cyanidin 3,3′,5-tri-*O*-glucoside. Compound **2**, **4** and **5** exhibited a molecular ion $[M + H]^+$ at *m/z* 611, along with a fragment ion at *m/z* 287 ($[M + H\text{-}162\text{–}162]^+$ loss of two glucose moieties), confirmed as cyanidin 3,5-*O*-diglucoside isomers published previously (Fischer et al., 2011; Hou et al., 2013; Hao et al., 2015). Compound **3** held a molecular ion $[M + H]^+$ at *m/z* 449 as well as a fragment ion at *m/z* 287 ($[M + H\text{-}162]^+$ loss of a glucose moiety), identified as cyanidin 3-*O*-glucoside by reference to previous report (Fischer et al., 2011; Hou et al., 2013; Hao et al., 2015). Compound **6** presented a molecular ion at *m/z* 433, together with a fragment ion at *m/z* 271 ($[M + H\text{-}162]^+$ loss of one glucose moiety, confirmed as pelargonidin 3-*O*-glucoside published previously (Mullen et al., 2008). Compounds **7** and **8** showed a similar parent ion at *m/z* 535, as well as a fragment at *m/z* 287 ($[M + H\text{-}162\text{–}86]^+$ loss of one glucose and one

Table 1
Qualification of phytochemical constituents by UPLC-Q-TOF-MS/MS in black hulless barley bran.

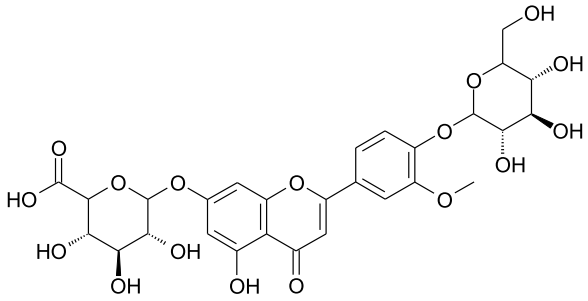
Peak no.	Rt (min)	$[M+H]^+$ (*m/z*)	Formula	Fragment ions	Identified compounds	20 %	40 %	80 %
#1	4.688	773.2142	C33H41O21	287	Cyanidin 3,3′,5-tri-*O*-glucoside	–	+	+
2	4.748	611.1701	C27H31O16	287	Cyanidin 3,5-*O*-diglucoside	–	+	+
3	5.231	449.1171	C21H21O11	287	Cyanidin 3-*O*-glucoside	–	+	+
4	5.261	611.1701	C27H31O16	287	Cyanidin-*O*-diglucoside	–	+	+
5	5.575	611.1802	C27H31O16	287	Cyanidin-*O*-diglucoside	–	+	–
6	5.942	433.1229	C21H21O10	271	Pelargonidin 3-*O*-glucoside	–	+	+
7	6.624	535.116	C24H23O14	287	Cyanidin 3-*O*-(6″-*O*-malonyl)-galactoside	–	+	+
8	7.393	535.116	C24H23O14	287	Cyanidin 3-*O*-(6″-*O*-malonyl)-galactoside	–	+	+
#9	8.219	783.1716	C35H43O20	287	Cyanidin 3,5-di-*O*-[6″-*O*-(malonyl)-galactoside]	–	+	–
#10	8.503	783.1716	C35H43O20	287	Cyanidin 3,5-di-*O*-[6″-*O*-(malonyl)-glucoside]	–	+	–
#11	8.785	621.1219	C27H25O17	287	Cyanidin 3-*O*-(3″,6″-*O*-dimalonyl)-glucoside	–	+	–
#12	9.554	621.1219	C27H25O17	287	Cyanidin 3-*O*-(3″,6″-*O*-dimalonyl)-glucoside	–	+	+
#13	9.898	549.1324	C25H25O14	287	Cyanidin 3-*O*-(6″-*O*-succinyl)-glucoside	–	+	–
#14	10.436	563.1505	C25H23O15	287	Cyanidin 3-*O*-(6″-*O*-succinyl)-glucuronide	–	+	+
#15	11.09	635.1276	C28H27O17	287	Cyanidin 3-*O*-(3″,6″-*O*-dimalonyl)-glucuronide	–	+	–
#16	11.518	635.1379	C28H27O17	287	Cyanidin 3-*O*-(3″,6″-*O*-dimalonyl)-glucuronide	–	+	–
#17	11.577	563.1505	C25H23O15	287	Cyanidin 3-*O*-(6″-*O*-succinyl)-glucuronide	–	+	–
#18	12.468	649.1435	C29H29O17	287	Cyanidin 3-*O*-(3″,6″-*O*-disuccinyl)-galactoside	–	+	+
#19	12.753	649.1435	C29H29O17	287	Cyanidin 3-*O*-(3″,6″-*O*-disuccinyl)-galactoside	–	+	+
20	6.434	463.1313	C22H22O11	301,286,258,229	Chrysoeriol-*O*-glucoside	–	+	–
#21	9.328	549.1324	C25H24O14	301,286,258	Chrysoeriol 7-*O*-(6″-*O*-malonyl)-galactoside	–	+	–
22	9.847	433.1187	C21H20O10	313,283	Isovitexin or vitexin	–	+	+
*23	10.476	449.1127	C21H20O11	287,153	Luteolin 7-*O*-glucoside	–	–	+
*24	10.568	463.0961	C21H18O12	287,153	Luteolin 7-*O*-glucuronide	–	+	+
#25	10.622	639.1653	C25H24O14	301,286,258	Chrysoeriol-*O*-glucoside-*O*-glucuronide	–	+	–
#26	11.136	639.1653	C27H26O18	477,301,286,258,229,153	Chrysoeriol 7-*O*-glucuronide 4′-*O*-glucoside	–	+	+
#27	11.246	653.1418	C27H24O19	301,286,258,229,153	Chrysoeriol 7-*O*-diglucuronide	–	–	+
#28	12.216	653.1313	C27H24O19	301,286,258,229,153	Chrysoeriol 7-*O*-diglucuronide	–		+
#29	12.274	609.1743	C26H24O17	463,301,286,258	Chrysoeriol-*O*-rhamnoside-*O*-glucoside	–	+	+
#30	12.476	639.1653	C27H26O18	477,301,286,258	Chrysoeriol-*O*-glucoside-*O*-glucuronide	–	+	+
#31	12.675	639.1705	C27H26O18	477,301,286,258	Chrysoeriol-*O*-glucoside-*O*-glucuronide	–	+	+
#32	12.723	577.1584	C26H24O15	301,286,258	Chrysoeriol 7-*O*-(6″-*O*-succinyl)-glucuronide	–	+	+
*33	12.734	463.1313	C21H18O12	301,286,258,229,183	Chrysoeriol 7-*O*-glucoside	–	+	+
*34	12.807	477.1062	C22H20O12	301,286,258,229,153	Chrysoeriol 7-*O*-glucuronide	–	+	+
#35	12.93	609.149	C27H28O16	477,301,286,258,229	Chrysoeriol-*O*-pentoside-*O*-glucuronide	–	–	+
#36	13.519	477.1062	C22H20O12	287,153	Luteolin 7-*O*-methylglucuronide	–	+	
#37	13.6	563.1116	C26H27O14	301,286,258	Chrysoeriol 7-*O*-(6″-*O*-succinyl)-glucoside	–	–	+
#38	13.918	549.1324	C25H24O14	463,301,286,258,229,153	Chrysoeriol 7-*O*-(6″-*O*-malonyl)-glucoside	–	+	+
#39	13.954	653.1679	C27H24O19	491,301,286,258	Chrysoeriol-*O*-methylglucuronide-*O*-glucoside	–	+	–
#40	14	563.1116	C26H27O14	301,286,258,229	Chrysoeriol 7-*O*-(6″-*O*-succinyl)-glucoside	–	+	+
#41	14.296	653.1783	C27H24O19	491,301,286,258	Chrysoeriol-*O*-methylglucuronide-*O*-glucoside	–		+
#42	14.37	491.121	C24H26O11	287,153	Luteolin 7-*O*-(6″-*O*-acetyl)-glucoside	–	+	+
*43	14.454	287.0577	C15H10O6	153	Luteolin	–	+	+
#44	14.656	491.121	C24H26O11	301,286,258,229,153	Chrysoeriol 7-*O*-methylglucuronide	–	+	+
#45	14.709	667.1954	C30H34O17	505,301,286,258	Chrysoeriol-*O*-acetylglucoside-*O*-glucoside	–	+	+
#46	14.851	667.206	C30H34O17	505,301,286,258	Chrysoeriol-*O*-acetylglucoside-*O*-glucoside	–	+	+
#47	15.306	753.1874	C33H36O20	447,301,286,258	Chrysoeriol-*O*-glucuronide-*O*-(6″-*O*-succinyl)-glucuronide	–	–	+
#48	15.307	667.1954	C30H34O17	505,301,286,258	Chrysoeriol-*O*-acetylglucoside-*O*-glucoside	–	+	+
#49	15.446	505.1364	C24H24O12	301,286,258,229	Chrysoeriol 7-*O*-(6″-*O*-acetyl)-glucoside	–	+	+
#50	15.858	577.1584	C26H24O15	301,286,258,229	Chrysoeriol 7-*O*-(6″-*O*-succinyl)-glucuronide	–	–	+
*51	15.899	301.0773	C16H12O6	286,258,229,153	Chrysoeriol	–	+	+

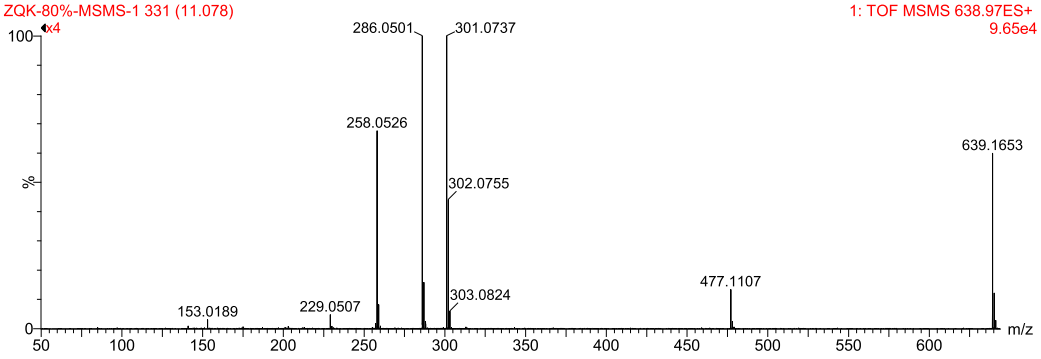
-, No detected. +, Detected. *, identified by reference compound. #, first identified in hulless barley.

malonic acid), certified as cyanidin 3-*O*-(6″-*O*-malonyl)-glucoside isomers (Viacava et al., 2017; Chen et al., 2012). During the grain color-changing and maturation stages, cyanidin 3-*O*-(6″-*O*-malonyl)-glucoside together with cyanidin 3-*O*-glucoside were significantly increased in the purple huless barley grains (Xu et al., 2022). Compounds **9** and **10** showed a similar parent ion at *m/z* 783, and a fragment at *m/z* 287 ($[M+H\text{-}162\text{–}162\text{–}86\text{–}86]^+$ loss of two glucose moieties and two malonyl moieties), affirmed as cyanidin 3,5-di-*O*-[6″-*O*-(malonyl)]-glucoside isomers (Tatsuzawa et al., 2011). Compounds **11** and **12** showed a similar parent ion at *m/z* 621, along with a fragment at *m/z* 287 ($[M+H\text{-}162\text{–}86\text{–}86]^+$ loss of one glucose moiety and two malonyl moieties), identified as cyanidin 3-*O*-(3″,6″-*O*-dimalonyl)-glucoside isomers, which structure was confirmed by Chen et al. (2012) and Nakayama et al. (1997). Compounds **13** showed a parent ion at *m/z* 549, as well as a fragment at *m/z* 287 ($[M+H\text{-}162\text{-}100]^+$ loss of one glucose moiety and one succinyl moiety), confirmed as cyanidin 3-*O*-(6″-*O*-succinyl)-glucoside which was also identified in *Phragmites australis* (Fossen and Øyvind, 1998). Compounds **14** and **17** showed a same parent ion at *m/z* 563, together with a fragment at *m/z* 287 ($[M+H\text{-}176\text{-}100]^+$ loss of one glucuronyl moiety and one succinyl moiety), established as cyanidin 3-*O*-(6″-*O*-succinyl)-glucuronide isomers. Compounds **15** and **16** showed a same parent ion at *m/z* 635, and a fragment at *m/z* 287 ($[M+H\text{-}176\text{-}86\text{-}86]^+$ loss of one glucuronyl moiety and two malonyl moieties), identified as cyanidin 3-*O*-(3″,6″-*O*-dimalonyl)-glucuronide isomers. Compounds **18** and **19** showed a same parent ion at *m/z* 649, as well as a fragment at *m/z* 287 ($[M+H\text{-}162\text{-}100\text{-}100]^+$ loss of one glucose moiety and two succinyl moieties), confirmed as cyanidin 3-*O*-(3″,6″-*O*-disuccinyl)-glucoside isomer. Up to present, there is no identification details about **Compounds 1, 14-19** in published papers.

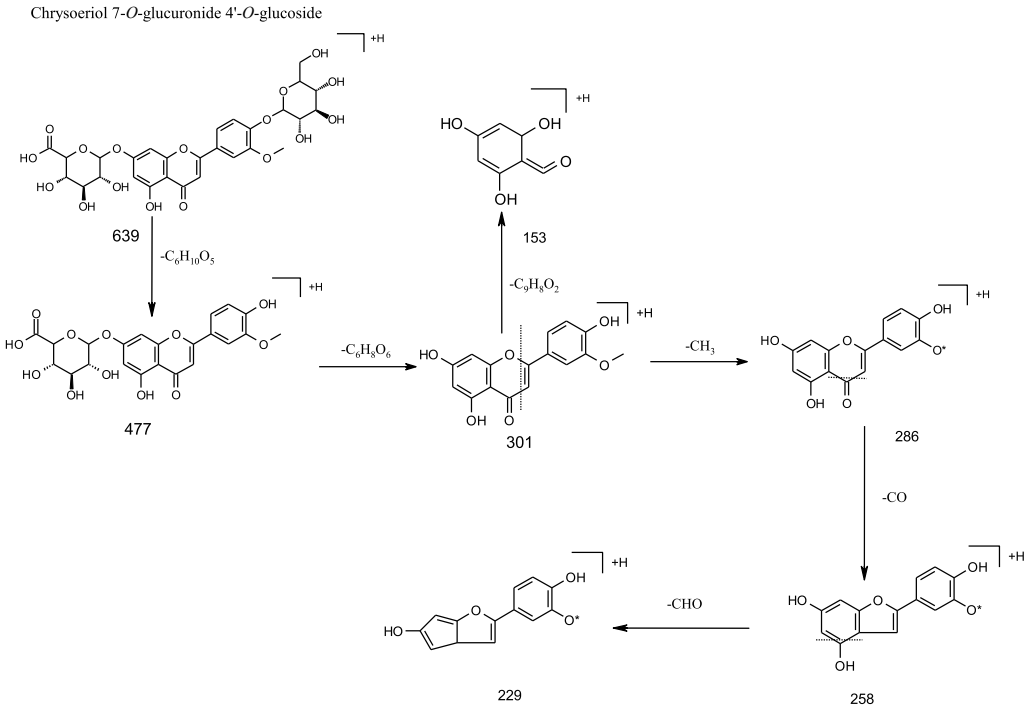
Compounds number **23**, **24**, **33**, **34**, **43** and **51** were unambiguously established as chrysoeriol-7-*O*-glucoside, luteolin-7-*O*-glucoside, luteolin 7-*O*-glucuronide, chrysoeriol 7-*O*-glucuronide, luteolin as well as chrysoeriol, respectively, based on the comparisons of MS fragment characteristics with authentic standards, and combination with previous

a. Chrysoeriol 7-*O*-glucuronide 4'-*O*-glucoside





639.1653,477,301,286,258,229,153

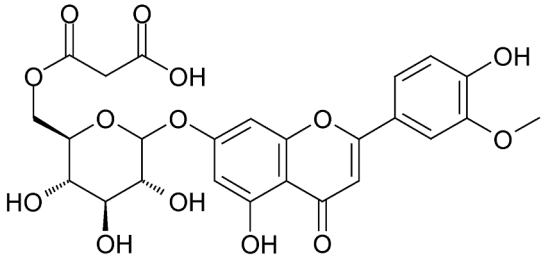


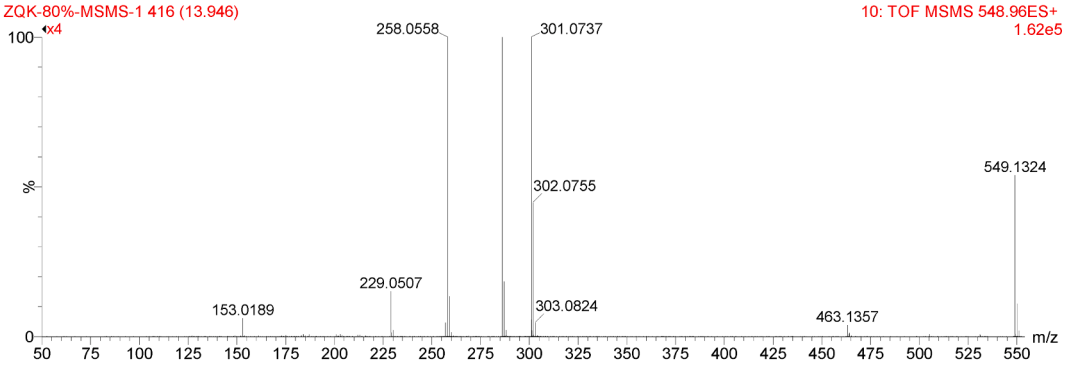
studies (Petar et al., 2007; Kumar et al., 2017). Compounds number **20**–**22**, **25**–**32**, **35**–**42**, and **44**–**50** were preliminary identified by operating a combination of MS spectra in the published references.

Compound **22** exhibited the parent ion $[M + H]^+$ at *m/z* 433, and fragment ion at *m/z* 313 ($[M + H\text{-}C_3H_6O_3]^+$), 283 ($[M + H\text{-}C_4H_8O_4]^+$). Through comparison with corresponding MS data, it was preliminary

Fig. 1. The chemical structure, characteristics of secondary ion mass spectrum in positive, and fragmentation patterns of Chrysoeriol 7-*O*-glucuronide 4′-*O*-glucoside (a) and Chrysoeriol 7-*O*-(6″-*O*-malonyl)-glucoside (b).

b. Chrysoeriol 7-*O*-(6″-*O*-malonyl)-glucoside





549.1324, 463,301,286,258,229,153

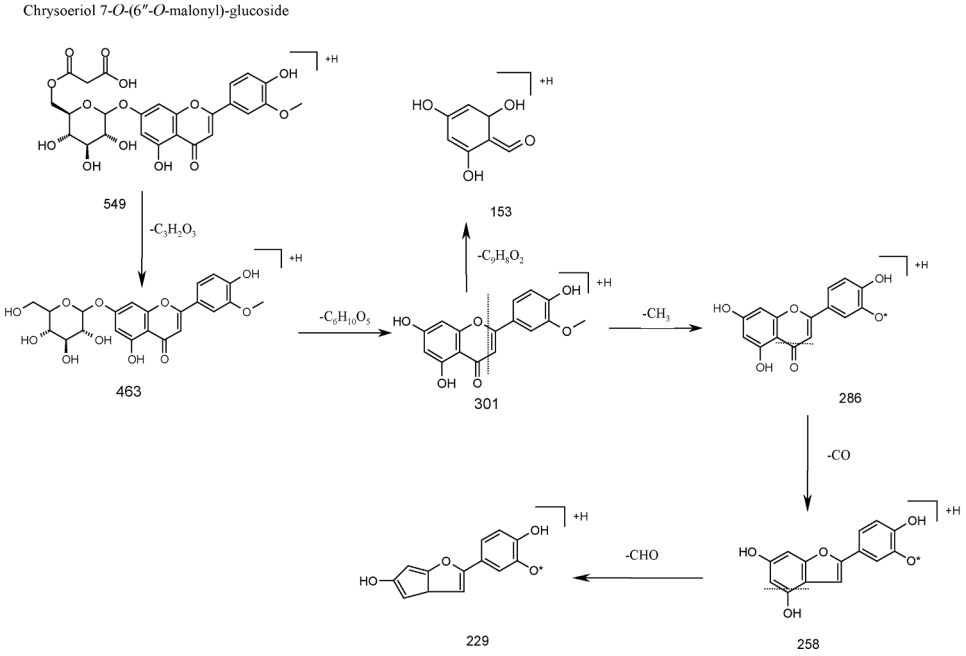


Fig. 1. (*continued*).

identified as isovitexin or vitexin (Peng et al., 2005). Compound **51** showed positive molecular ion $[M + H]^+$ at *m/z* 301, together with fragment into ions at *m/z* 286 ($[M + H\text{-}CH_3]^+$), 258 ($[M + H\text{-}CH_3\text{-}CO]^+$), 229 ($[M + H\text{-}CH_3\text{-}CO\text{-}HCO]^+$), 153(RDA 1/3). Furthermore, it was identified as chrysoeriol by using authentic standards, which was also reported by Petar et al. (2007). These fragments allow us to rapidly distinguish the derivatives of chrysoeriol in chemical profiling. Compounds **20**–**21**,**25**–**32**,**35**–**41**, **44**–**50** showed the same fragment ion at 301, 286, 258, which indicated that they were chrysoeriol derivatives. Compound **20** showed the parent ion $[M + H]^+$ at *m/z* 463, and the MS^2 ions at *m/z* 301 ($[M + H\text{-}162]^+$ loss of one glucose moiety), 286, 258, 229, which indicated that it was chrysoeriol -*O*-glucoside isomer. Compounds **21** and **38** showed the deprotonated $[M + H]^+$ ion with *m/z* 549, further fragmented into ions at *m/z* 301 ($[M + H\text{-}162\text{-}86]^+$ loss of one glucose moiety and one malonyl moiety), thus they were identified as chrysoeriol 7-*O*-(6″-*O*-malonyl)-glucoside isomers (Conrad et al., 2009). Compounds **25**, **26**, **30** and **31** presented the same parent ion $[M + H]^+$ at *m/z* 639, demonstrating their possibility as isomers. They

produced MS^2 ions at *m/z* 477 ($[M + H\text{-}162]^+$ loss of one glucose molecule), 301 ($[M + H\text{-}162\text{-}176]^+$ loss of one glucose molecule and one glucuronyl group), so they were comfirmed as chrysoeriol-*O*-glucoside-*O*-glucuronide isomer. Compounds **27** and **28** showed the same parent ion at *m/z* 653, along with fragment ions at *m/z* 477 ($[M + H\text{-}176\text{-}176]^+$ loss of two glucuronyl groups), and 286, 258, 229, 153. so they were recognized as chrysoeriol-7-*O*-diglucuronide isomers. Compound **29** presented the parent ion at *m/z* 609, fragment ion at *m/z* 463 ($[M + H\text{-}146]^+$ loss of one rhamnoside group) and 301 ($[M + H\text{-}146\text{-}162]^+$ loss of one rhamnoside group and one glucoside group). Based on the fragments and relevant literature (Petar at al., 2007), compound **29** was identified as chrysoeriol-*O*-rhamnoside-*O*-glucoside. Compounds **35** showed the parent ion at *m/z* 609, together with fragment ion at *m/z* 477 ($[M + H\text{-}132]^+$ loss of one pentoside group), and 301 ($[M + H\text{-}132\text{-}176]^+$ loss of one pentoside group and one glucuronyl group), which indicated it was chrysoeriol-*O*-pentoside-*O*-glucuronide. Compounds **37** and **40** showed same parent ion at *m/z* 563, as well as fragment ion at *m/z* 301 ($[M + H\text{-}162\text{-}100]^+$ loss of one glucose molecule and one succinyl group), which indicated they were chrysoeriol 7-*O*-(6″-*O*-succinyl)-glucoside isomers. Compounds **39** and **41** showed same parent ion at *m/z* 653, along with fragment ion at *m/z* 491 ($[M + H\text{-}162]^+$ loss of one glucose molecule), 301 ($[M + H\text{-}162\text{-}176\text{-}14]^+$ loss of one glucuronyl group as well as a methyl group from fragment ion at *m/z* 491), indicating they were chrysoeriol-*O*-methylglucuronide-*O*-glucoside isomers. Compound **44** showed fragmented into ion at *m/z* 301 ($[M + H\text{-}176\text{-}14]^+$ loss one glucuronyl group and a methyl group), indicated it was chrysoeriol-*O*-methylglucuronide. Compounds **45**,**46** and **48** showed same parent ion at 667, and fragment ions at *m/z* 505 ($[M + H\text{-}162]^+$ loss of one glucose molecule), and 301 ($[M + H\text{-}162\text{-}162\text{-}42]^+$ loss of two glucose moieties and one acetyl moiety), which indicated they were chrysoeriol-*O*-acetylglucoside-*O*-glucoside isomers. Compound number **47** showed parent ion at 753, as well as fragment ions at *m/z* 447 ($[M + H\text{-}132\text{-}132\text{-}42]^+$ (loss of two pentose groups along with one acetyl group), 301 ($[M + H\text{-}132\text{-}132\text{-}42\text{-}146]^+$ loss of two pentose groups, one acetyl grouploss and one rhamnose moiety), which indicated it was chrysoeriol-*O*-digpentose-(6″-*O*-acetyl)-rhamnose. Compound **49** showed the ions of fragment was at *m/z* 301 ($[M + H\text{-}162\text{-}42]^+$, lossing of one glucose molecule as well as one acetyl group), which indicated it was chrysoeriol 7-*O*-(6″-*O*-acetyl)-glucoside. Compound **50** showed ions of fragment was at *m/z* 301 ($[M + H\text{-}176\text{-}100]^+$, lossing of one glucuronyl group and one succinyl group), which indicated it was chrysoeriol 7-*O*-(6″-*O*-succinyl)-glucuronide.

Compound **43** was identified as luteolin, using authentic standards. It showed positive molecular ion [M + H]- at *m/z* 287 and further fragmented into ion at *m/z* 153, similar to the result reported by Kumar et al. (2017). Compounds **36** and **42** showed the ions of fragments at *m/z* 287 and 153, suggesting they were luteolin derivatives. As the fragment of compound **36** was at *m/z* 287 ($[M + H\text{-}176\text{-}14]^+$ loss of one glucuronyl group as well as one methyl group, it was further established as luteolin 7-*O*-methylglucuronide. Compound **42** exhibited the pseudomlecular ion at *m/z* 491, together with the fragment ion at *m/z* 287 ($[M + H\text{-}162\text{-}42]^+$ losing of one glucoside group and one acetyl group), so it was vertified as luteolin 7-*O*-(6″-*O*-acetyl)-glucoside. However, identification details about compounds **25–28, 30–32, 35–37, 39–42, 44–50** were not found in published papers. And the compounds **1, 9–19, 21, 25–32, 35–42, 44–50** were first identified in hulless barley.

3.2. Quantification

Chrysoeriol, chrysoeriol 7-*O*-glucouronid, luteolin as well as luteolin 7-*O*-glucouronid were further quantified using UPLC, which exhibited as the higher content than other flavonoids in hulless barley in our research and also proved in other literature (Xiang et al., 2021). As shown in Table 2, the four flavonoids were all not detected in E20%, and higher content in E80% than that of in E40%. This was likely that the polarity of these four flavonoids were closer to that of E80%. As widely distributed in the plant kingdom, Luteolin and its glycosides were proved to possess the potential activities to antioxidant, anti-inflammatory, antimicrobial as well as anticancer (Bangar et al., 2023; Wang et al., 2021). Compared with other cereal (Tiozon et al., 2022; Suchowilska et al., 2020), black hulless barley exhibited higher content of luteolin (1.79 mg/g in E40%, and 6.82 mg/g in E80%) than that of rice, sorghum and wheat (0.5–1 mg/100 g, 0–18.2 mg/100 g, 3.1–4.14 mg/100 g, respectively). The higher content of flavonoid in hulless barley might be correlated with its cultivated place-Tibetan Plateau (>4,000 m above sea level), which is characterized by the high UV-B radiation (about 65 kJ m^{-2} in the summer), low temperatures (average yearly temperature 7.6 °C), and low barometric pressure (about 650 mbar) (Norsang et al., 2009). On the evolution of the downstream phenylpropanoid pathway, most plants have adopted flavonoids as the major line of defense in resisting UV-B radiation (Zeng et al., 2019). Chrysoeriol, a 30-O-methoxy flavone, is a methoxy derivative of luteolin observed in numerous plants and observed as several derivatives. (Boro et al., 2023; Bangar et al., 2023). It has the anticancer, anti-inflammatory, antibacterial, antifungal, anti-osteoporosis, anti-insecticide, and neuroprotective actions (Boro et al., 2023). However, little information about the quantitative analysis of chrysoeriol in other cereal in published papers.

As an important group of water-soluble pigments in plants, anthocyanins would produce red, purple, blue as well as intermediate hues in plant tissues (Clifford, 2000). Another, anthocyanins is significant because of their antioxidant, anticancer and antidiabetes activities and human nutrition (Konczak and Zhang, 2004; Stintzing and Carle, 2004). From Table 2, TAC showed the trend of E40% > E80% > E20%, which indicated that large amount of anthocyanins in black hulless barley bran were resolved in 40 % ethanol. This result was accordance with the UPLC-QTOF-MS/MS result (Table 1), no anthocyanidins and flavonoids was detected in E20%, 19 anthocyanidins and 24 flavonoids were found in E40%, 10 anthocyanidins and 28 flavonoids were found in E80%.

Being recognized as a major group of phytochemicals, phenolic compounds contribute largely to the antioxidant capacity in cereals (Van Hung, 2016; Šimić et al., 2019). The hulless barley was also a rich cereal of phenolic compounds (Zeng et al., 2019; Ge et al., 2020). In present study, the TPC and TFC in E40% were 237.83 and 125.16 mg/g,

Table 2
Quantification of extracts in black hulless barley bran.

Flavonoid compounds	Equation	R^2	Linear range (μg/mL)	Content (mean ± SD, mg/g)		
				E20%	E40%	E80%
Luteolin 7-*O*-glucouronid	$y = 11.245\,x + 2.217$	0.9999	2–200	0	8.56 ± 0.04b	9.45 ± 0.04a
Chrysoeriol 7-*O*-glucouronid	$y = 9.3088\,x - 64.583$	0.9994	2.5–225	0	29.97 ± 0.13b	132.78 ± 0.61a
Luteolin	$y = 10.11\,x - 7.34$	0.9999	2–200	0	1.79 ± 0.01b	6.82 ± 0.03a
Chrysoeriol	$y = 12.366\,x + 1.0054$	0.9999	2.46–246	0	3.20 ± 0.02b	28.67 ± 0.04a
TAC	$y = 3.4613\,x - 13.562$	0.9999	7.81–1000	8.53 ± 0.78c	176.26 ± 7.56a	31.32 ± 0.45b
TPC	$y = 0.0069\,x + 0.0273$	0.9981	3.31–212	190.80 ± 3.79b	237.83 ± 1.28a	133.18 ± 5.22c
TFC	$y = 0.0013\,x + 0.0037$	0.9985	1.56–200	107.55 ± 4.73b	125.16 ± 5.13a	63.97 ± 5.45c

Different lowercase superscripts within the same column indicate significant difference ($p < 0.05$).

respectively. The total phenolic content was significantly affected by cultivars, milling conditions and milling fractions (Šimić et al., 2019; Moza and Gujral, 2017). From Table 2, the TPC and TFC showed same trend of E40% > E20% > E80%. Ethanol is a polar-protic solvent which is preferential to extract low molecular weight compounds, such as glycosylated and non-glycosylated phenolic compounds (Yusoff et al., 2022). However, different ethanol concentration exhibited different capacity on extraction content and type of polyphenols. Bai (2014) also reported that 40 % ethanol as the extraction solvent could obtained higher content polyphenols in apple pomace than using 20 % and 80 % ethanol. Furthermore, as shown in Table 2, not a single flavonoid or phenolic acid compound was identified with UPLC-MS/MS, E20% exhibited higher TPC and TFC than those of E80%. In our previous study (Deng et al., 2021), ferulic acid was the predominant phenoilc acid in hulless barley, ranging from 266.6 to 760.64 μg/g. Furthermore, Ge et al. (2020) identified 156 phenolic substances, including monophenol, phenolic acids, flavonoids and other polyphenols. And the contents of chlorogenic acid (16.898 μg/g), quercitrin (184.093 μg/g), vitexin (253.012 μg/g) were more higher than other quatified polyphenols in black hulless barley. These implied that there were abundance of flavonoid or polyphenols compound in E20%.

3.3. Antioxidant properties

Antioxidants are believed as the essential for the body defense system against oxidative stress (Kancheva and Kasaikina, 2013). Thus antioxidant capacity has become a desirable and essential quality characteristic of plant. In present study, the antioxidant activity of three extracts were examined through the ferric reducing antioxidant potential (FRAP) tests, and DPPH, ABTS radical scavenging assays.

The high correlation coefficients between absorbance and tested concentrations of E80%, E40%, E20% and trolox (0.9956, 0.9992, 0.9985 and 0.9999), demonstrated that the reducing power of the three samples together with trolox was dose-dependent. As shown in Fig. 2**a**, the reducing power followed the trend as trolox > E40% > E20% > E80%. The scavenging effect on DPPH radical was also in a concentration dependent manner (Fig. 2**b**). The DPPH IC_{50} values, the concentration of sample to inhibition 50 % of the radical, were 15.02 ± 0.22, 99.52 ± 2.36, 45.49 ± 0.62, 53.93 ± 0.47 μg/mL for trolox, E80%, E40%, E20%, respectively ($P < 0.01$). The sequence of scavenging effect was trolox > E40% > E20% > E80%. This result was consistent with reducing power analysis. And the three extracts were all exceed 80 % scavenging effect on DPPH radical within a certain concentration range. As shown in Fig. 2**c**, the scavenging effect all samples on ABTS radical was also concentration dependent. The IC_{50} values of trolox, E80%, E40% and E20% were 44.40 ± 1.06, 47.80 ± 1.60, 25.60 ± 1.33 and 28.02 ± 0.91 μg/mL, respectively ($P < 0.01$). The sequence of scavenging effect was trolox > E40% > E20% > E80%. The similar trend were exhibited in reducing power and DPPH assays (Fig.a-b). And the three extracts were all exceed 90 % scavenging effect on ABTS radical within a certain concentration range.

The reaction mechanism of 2,2-azinobis (3-ethyl-benzothiazoline-6-sulphonic acid) (ABST), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant potential (FRAP) tests all belong to the single electron transfer mode (Kancheva and Kasaikina, 2013), which maybe the main reason for the phenomenon that three extracts have similar change trends in antioxidant activity: E40% > E20% > E80%. Another, the same trends were discovered in TPC and TFC (Table 2). Phenolic compounds are considered as the major antioxidants in cereals (Van Hung, 2016; Ge et al., 2020). So phenolics and flavonoids might be the major antioxidant compounds in hulless barley samples.

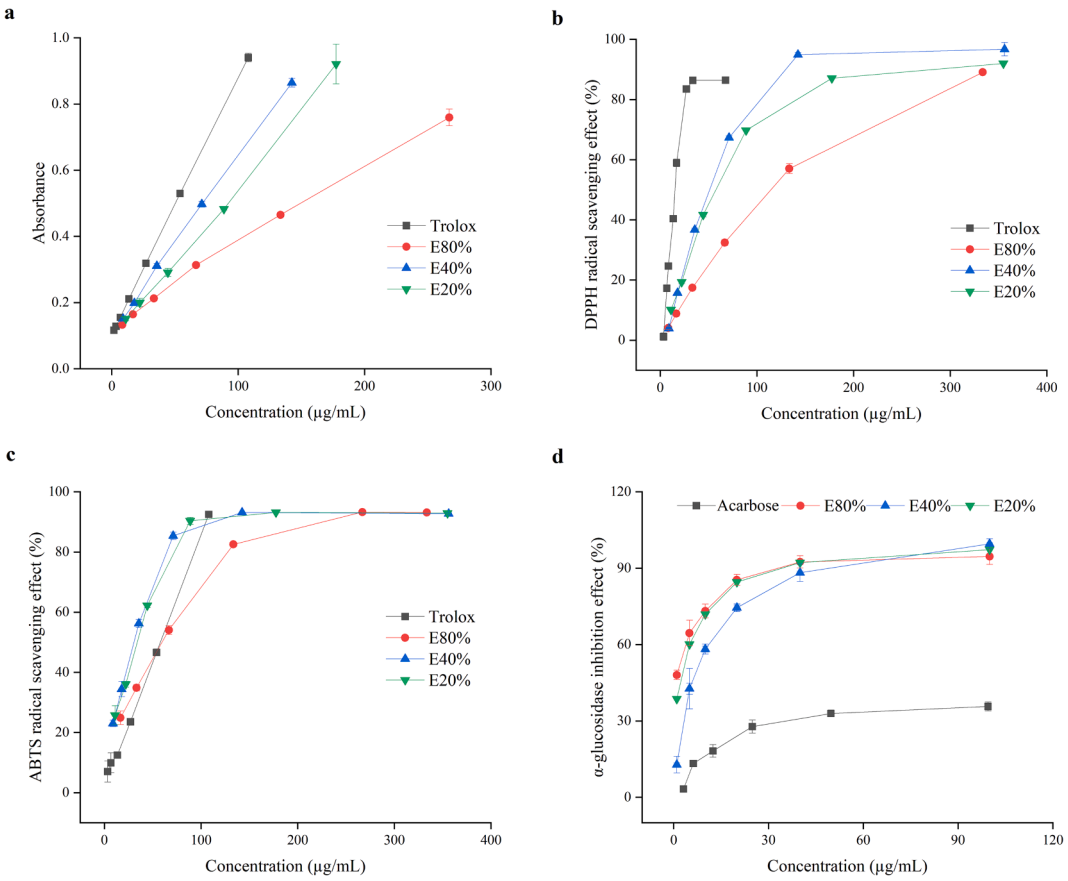


Fig. 2. The antioxidant activity (a, reducing power. b, DPPH. c, ABTS.) and α-glucosidase inhibition effect of extracts in black hulless barley bran (d).

3.4. α-glucosidase inhibition effect assay

Due to gastro-intestinal intolerability, side effects as well as high cost, the use of commercial α-glucosidase inhibitors (acarbose, miglitol and voglibose) is restricted **(Andrea and Davide, 2014)**. Plant extracts, such as cranberry (Apostolidis et al., 2006), tea polyphenols (Kan et al., 2021), brown rice bound phenolics (Ye et al., 2022) were investigated to have strong α-glucosidase inhibitory effect, potentially demonstrating their efficacy in effective management of obesity and diabetes mellitus. Our result also showed obvious dose-dependent manner when referred to inhibition effect on α-glucosidase. The IC50 values were 188.42 ± 36.63, 1.47 ± 0.19, 5.99 ± 1.81, 2.33 ± 0.34 μg/mL for acarbose, E80%, E40%, E20%, respectively. The IC50 values of three sample were significantly lower than those of acarbose ($p < 0.01$). The inhibition effect followed the pattern as: E80% > E20% > E40% > acarbose. The results manifested that compared with acarbose, these three samples were preferable α-glucosidase inhibitors *in vitro*, which containing exceed 90 % α-glucosidase inhibition effect within a certain concentration range. Another, from the Table 1, 0, 24 and 27 flavonoids were identified in E20%, E40%, E80%, respectively. But E80% contained lower content of TFC. Yang et al. (2021) reported that the number of hydroxyl groups on the A-ring might of flavonoids served as the most effective α-glucosidase inhibitor. So it was hypothesized that there were more specific flavonoid substances, which had the good effect on inhibition of α-glucosidase, in E80% portion.

3.5. Correlation analysis

The high negative correlations were exhibited between the TPC and the antioxidant activities (presented by IC50 value, which smaller means stronger antioxidant capacity) measured by reducing power, DPPH, ABTS (r = -949, r = -0.948, r = -0.820, $p < 0.01$,Table 3), representing that the higher TPC, the stronger antioxidant activities, in agreement with our previous studies on hulless barley (Deng et al., 2021). Similarly, TPC in colored barley were found to be the main contributors of DPPH and ABTS free radical scavenging activities (Suriano et al., 2018). Plant polyphenols are multifunctional, which could be served as hydrogen donating antioxidants, reducing agents, as well as singlet oxygen quenchers (Rice-Evans et al., 1996).

Additional, the high negative correlations were found between TFC and the antioxidant activities (presented by IC50 value) inluding reducing power, DPPH, ABTS (r = -971, r = -0.981, r = -0.955, $p < 0.01$), suggesting that flavonoids were also the important ingredients of antioxidant activity in huless barley. Antioxidant activities of plants were closely associate with their polyphenols and flavonoids (Scalbert et al., 2005). The antioxidant activity of the flavonoids relies on their capability, which contribute protons and electrons to resist the impact of energetic oxidants, for instance free radicals (Martins et al., 2004).

The positive correlations between α-glucosidase inhibition effect and TAC, TPC and TFC were displayed in Table 3, indicating that total anthocyanin, total phenolic compounds and total flavonoids were not significant effective on a-glucosidase inhibitory activity. It was reported that phenolic phytochemicals from plant sources might be efficiently a-glucosidase inhibitors (Apostolidis and Lee, 2010; Wu and Xu, 2014; Nguyen et al., 2022). The catalytic activity of α-glucosidase could be reduced because of the reversible combination of polyphenols and glucosidase (Wang, 2017). However, the significant inhibition effect of TAC, TPC and TFC on α-glucosidase were not found in present study. β-glucan have the potential to lower the synthesis of cholesterol and reduce the concentration of low-density blood lipid, and hulless barley is the richest cereal in terms of β-glucan content (Zhang et al., 2019). But Tian et al. (2013) reported that the highland barley extract had a better effect than single β-glucans on hypoglycemic activity. Bellesia and Tagliazucchi (2014) pointed that the binding force and stability of polyphenols and a-glucosidase are different, largely depending on the structure of polyphenols, including structure of parent nucleus, type and number of substituent, and binding site. Yao et al. (2022) reported that hyperoside and scoparone might be the main active substance in hulless barley on lipid-lowering effects. So β-glucans, hyperoside, scoparone and other flavonoids might be the main activity compounds on α-glucosidase inhibitory activity.

No significantly negative correlations were obtained among

Table 3
Pearson's correlation coefficients among TAC, TPC, TFC, antioxidant activity, inhibition of a-glucosidase and four flavonoids in extracts of black huless barley bran.

	TAC	TPC	TFC	Reducing power	DPPH (used IC50 value, μg/mL)	ABTS (used IC50 value, μg/mL)	Inhibition of a-glucosidase (used IC50 value, μg/mL)	Luteolin 7-*O*-glucouronid	Chrysoeriol 7-*O*-glucouronid	Luteolin	Chrysoeriol
TAC	1	0.758*	0.624	−0.542	−0.518	−0.472	0.886**	0.53	−0.188	−0.142	−0.291
TPC		1	0.978**	−0.949**	−0.948**	−0.920**	0.842**	−0.145	−0.779*	−0.750*	−0.841**
TFC			1	−0.971**	−0.981**	−0.955**	0.776*	−0.318	−0.870**	−0.849**	−0.916**
Reducing power				1	0.994**	0.991**	−0.705*	0.415	0.923**	0.895**	0.949**
DPPH (used IC50 value, μg/mL)					1	0.988**	−0.688*	0.446	0.937**	0.915**	0.964**
ABTS (used IC50 value, μg/mL)						1	−0.644	0.48	0.947**	0.923**	0.965**
Inhibition of a-glucosidase (used IC50 value, μg/mL)							1	0.219	−0.432	−0.399	−0.522
Luteolin 7-*O*-glucouronid								1	0.728*	0.760*	0.653
Chrysoeriol 7-*O*-glucouronid									1	0.993**	0.989**
Luteolin										1	0.988**
Chrysoeriol											1

**$p < 0.01$ (2-tailed). *$p < 0.05$ (2-tailed).

antioxidant activities and the four compounds, including luteolin, luteolin 7-*O*-glucouronid, chrysoeriol and chrysoeriol 7-*O*-glucuronide, suggesting those four flavonoids might make little contributions to the antioxidant activities in hulless barley. The similar results were perceived in our previous study (Deng et al., 2021). In scavenging the DPPH radicals, earlier reports found that chrysoeriol was inactive, and chrysoeriol glycoside also exhibited not very efficient (Mishra et al., 2003). And luteolin 7-*O*-*β*-glucuronide was reported not relatively effective radical scavenger (Özgen et.al., 2011). Although luteolin and its derivatives feature a conjugated system, their antioxidant activity is generally lower compared to other flavonoids, particularly flavonols. This reduced activity is attributed to the absence of the 3-OH group, which eliminates a potential active site (Charlton et al., 2023).

Significantly positive correlations were found between luteolin, luteolin 7-O-glucouronid, chrysoriol, chrysoeriol 7-O-glucouronid. Luteolin, commonly named 3′,4′,5,7-tetrahydroxyflavone, is a flavone-based polyphenol naturally found as glycosides, with a phenylpropanoid biosynthesis route (Bangar et al., 2023). As a 30-O-methoxy flavone, chrysoeriol is a chemically derived product of luteolin (Aboulaghras et al., 2022).

4. Conclusion

By employing UPLC-QTOF-MS/MS, fifty-two constituents comprised 19 anthocyanidins and 32 flavonoids in black hulless barley bran fractions were characterized and identified. Among them, luteolin, luteolin 7-*O*-glucouronid, chrysoeriol and chrysoeriol 7-O-glucouronid were quantified by using UPLC-DAD, and they were all not detected in E20%, and higher content in E80% than that of in E40%. Another, the stronger antioxidant activity was obtained under higher TPC and TFC content, indicating that phenolics and flavonoids might be the key antioxidant compounds in black hulless barley bran. However, there were no significant correlation between TAC, TPC, TFC and α-glucosidase inhibitory activity. The current study could give improtant references for the utilization of black hulless barley bran, particularly for their consumption of anthocyanins and flavonoids of black barley bran.

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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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2024.105644.

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