# Blackberry (Rubus fruticosus L.) Fruit Extract Phytochemical Profile, Antioxidant Properties, Column Chromatographic Fractionation, and High-performance Liquid Chromatography Analysis of Phenolic Compounds

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***Abstract*—This groundbreaking study explores the untapped potential of blackberries, a member of the Rubus genus in the Rosaceae family, and sheds light on their remarkable health and medicinal properties. Unlike previous research conducted in other regions, this investigation focuses specifically on the blackberry fruit's phytochemical constituents, chromatographic fractionations, and antioxidant activities in the Koisinjaq and Erbil villages of Northern Iraq. The research unveils seven distinct fractions obtained through column chromatography, with Fractions 2 and 3,5 found to contain p-coumaric acid and rutin, respectively, while Fraction 2 also houses chlorogenic acid. The analysis reveals the impressive richness of the methanolic blackberry extract in phenolic content (38.08 mg gallic acid equivalent/g dry weight [DW]), flavonoids (14.58 mg quercetin equivalent/g DW), flavonols (6.95 mg rutin equivalent/g DW), and anthocyanins (7.73 mg/kg DW), underlining the fruit's potent antioxidant activity. Furthermore, blackberries display exceptional ferric-reduction and metal-chelating capabilities, with 20.53 mg $FeSO_4$/g and 182.12 mg $Fe^{2+}$/g DW, respectively. Remarkably, blackberries also exhibit a remarkable ability to inhibit amylase activity (76.01%). These findings open up exciting prospects for utilizing blackberry fruit as a natural and potent source of phytochemicals and antioxidants in the food and pharmaceutical industries, promising transformative applications in health and well-being.**

***Index Terms*—Rubus fruticosusm, Phytochemical, Antioxidants, Column chromatography, Phenolic.**

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## I. Introduction

Plants play a significant role in the food industry because of their sensory and nutritional properties, serving as sources of antioxidants to maintain food quality. They are used in medicine as medicinal herbs, providing healthcare and disease prevention for much of the global population (Hama, et al., 2016). Natural antioxidants can be found abundantly in medicinal herbs, making them an excellent source for creating contemporary medications. As various ailments such as cancer, liver disease, and arthritis do not have allopathic solutions, medicinal herbs have played a significant role in modern medicine (Shibu Prasanth and Chandran, 2017). Fruits and vegetables provide a variety of tastes and have been linked to an increased quality of life, making them essential for human health (Soquetta, Terra, and Bastos, 2018). Berries, with their unique color, flavor, aroma, high vitamin and mineral content, and range of food service applications, are a vital part of the fruit kingdom (Okatan, 2020). Blackberries, belonging to the Rubus genus in the Rosaceae family, are extensively grown globally. These fruits are native to tropical America, primarily Colombia and Ecuador (Sanín, Navia, and Serna-Jiménez, 2020). The global commercial output of blackberries is estimated to be approximately 154,578 tons/year, with North America, Europe, and Asia being the primary producers (Monforte, et al., 2018). Blackberries comprise several small drupes on a 1–2.5 cm long receptacle and are recognizable by their dark crimson color and bitter flavor (Fig. 1) (Sanín, Navia, and Serna-Jiménez, 2020).

The attractive color and flavor of blackberries (Rubus fruticosus) have made them a popular fruit, and they may also offer health benefits to humans (Santos, et al., 2019). Blackberries are rich in various bioactive

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Fig. 1. Blackberry (Rubus fruticosus L.) fruit.

compounds, such as flavonoids, phenolic acids, tannins, vitamins, and antioxidants (Kitrytė, et al., 2020). The fruit of blackberries contains phenolic compounds such as anthocyanins, flavonols, chlorogenic acid, and procyanidins that have a positive impact on human health (Zafra-Rojas, et al., 2018). Blackberries have been used medicinally in Europe since the 16$^{th}$ century, where they were used for treating eye and mouth diseases (Oszmiański, et al., 2015). Various studies have suggested that consuming blackberries regularly can be beneficial to human health, especially in preventing and treating chronic degenerative disorders (Padilla-Jimenez, et al., 2019). Thus, this study aimed to establish an experimental set-up to extract and then fractionate the fruits, to isolate and explain new/novel phytochemical content. Thus, this study aimed to establish an experimental set-up to extract and then fractionate the fruits, isolate and explain new/novel phytochemical content, evaluate the antioxidant activity of the fraction, and identify potential new bioactive compounds or drugs.

## II. Materials and Methods

### *A. Chemicals*

Only analytical-grade chemicals and solvents were used in this study. Silica gel for column chromatography (70–230 mesh ASTM), thin-layer chromatography (TLC), aluminum sheets 20 × 20 cm (60 F254), aluminum chloride, sodium acetate trihydrate, potassium ferricyanide, iodine, phenanthroline monohydrate, ferrous sulfate heptahydrate, dipotassium hydrogen phosphate, sodium carbonate, Folin-Ciocalteu's phenol reagent, sodium hydroxide, trichloroacetic acid, ferric chloride trihydrate, potassium hydroxide, and $HCO_2H$ was purchased from Merck (Germany). Methanol, ethanol, hexane, and sodium dihydrogen phosphate were got from Carlo Erba in Sabadell (Spain). Quercetin and ethyl acetate were purchased from Sigma-Aldrich (Germany). Gallic acid dry was supplied by ISOLAB GmbH. Rutin trihydrate was got from (Dr Ehrenstorfer™).

### *B. Plant Acquisition*

In October 2020, villagers in Northern Iraq's Koy Sanjaq/Erbil area (Fig. 2) gathered blackberries (Rubus fruticosus L.) fruit. The fruit was washed, dried in the shade, and then ground into a fine powder in an electric grinder. The powder was then sealed in airtight vials and kept for future analysis.

### *C. Plant Extraction*

Using maceration achieved success in extracting plant components. The powder material was extracted at room temperature 3 times for 24 h using 75 ml of MeOH. Whatman filter paper No. 1 was used to filter the extract and the filtrate was concentrated under decreased pressure and at 40°C. Hexane was used to remove the fat after the evaporation of the extract. Dry methanolic extract (3.44 g) was kept at 4–6°C for further analysis.

### *D. Total Phenolic Content (TPC)*

The TPC extract was determined by altering the Folin–Ciocalteu procedure (AkyÜz, et al., 2020). A 1.5 mL of distilled water was added to 0.1 mL of extract (3 mg/mL MeOH) and vigorously agitated for 5 min with 0.1 mL of Folin–Ciocalteu reagents, followed by 1.5 mL of sodium carbonate, 10% (w/v). After 60 min in the dark, the mixture's absorbance was determined at 765 nm using a Thermo Scientific GENESYS 10S ultraviolet-visible spectrophotometer (USA). A regression equation derived from the gallic acid standard calibration curve (Fig. 3a) was used to calculate the extract's TPC equivalents (mg gallic acid equivalent [GAE]/g dry weight [DW]).

### *E. Total Flavonoid Content (TFC)*

The aluminum chloride colorimetric technique was used to determine the flavonoid content of fruit extract with minimal development (Iqbal, Salim, and Lim, 2015). In total, 0.2 mL of extract (3 mg/mL) was combined with 1 mL of 5% $AlCl_3$ solution, followed by 0.1 mL of 1.0 M $CH_3COOK$ solution and 2.7 mL of MeOH then allowed to stand for 60 min. The absorbance was measured at 420 nm. A calibration curve (Fig. 3b) was used to compute the TFC, expressed as (mg quercetin equivalent [QE]/g DW).

### *F. Total Flavonol Content (TF)*

TF was quantified using a colorimetric approach using aluminum chloride (Binici, ŞAT, and Aoudeh, 2021), which was significantly changed. A standard calibration curve for this method was generated using rutin. A 0.5 mL solution of the extract (3 mg/mL) was added to a test tube, followed by 0.5 mL of 2% aluminum chloride and 5% sodium acetate (6 mL). After constant stirring, all tubes were incubated for 150 min at room temperature in a dark place. The absorbance was measured for the reference (Fig. 3c) and sample at 440 nm to get mg rutin equivalent (RE)/g DW values for the results.

### *G. Total Anthocyanin Content (TAC)*

The differential pH approach determined the amount of monomeric anthocyanin in its totality (Sutharut and Sudarat, 2012). The anthocyanin concentration of blackberry extract was measured by diluting it with potassium chloride (0.025 M) and sodium acetate (0.40 M) solutions and then

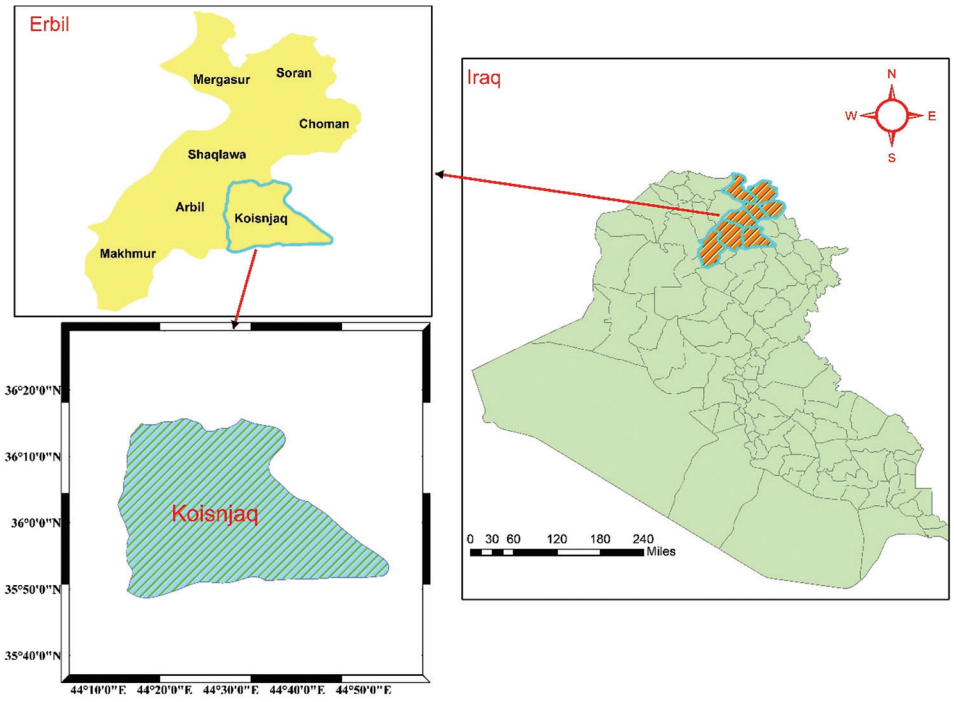


Fig. 2. GIS Map: Iraqi map, Erbil governorate location, and Koysinjaq study area location.

adjusting the pH with HCl to 1.0 or 4.5 as appropriate. The cyanidin-3-glucoside standard was used for absorbance measurement against a distilled water blank. The absorbance of each diluted solution was measured at two wavelengths, 520 nm, and 700 nm.

Calculation of absorption (A):

$$A = (A520 - A700)\ \text{pH}\ 1.0 - (A520 - A700)\ \text{pH}\ 4.5$$

The following formula was used to calculate the absorption (A):

$$\text{Anthocyanin extraction yield (mg/g)} = \frac{A \times MW \times DF \times V \times 1000}{\varepsilon \times L \times wt}$$

Where;

DF=Dilution factor, MW=Molar mass of cyanidin-3-glucoside (449.2 g/mol), ε=molar extinction coefficient of cyanidin-3-glucoside (26900 L/mol.cm), V=Extract volume (L), L=Sample cell Length (cm), 1000=Conversion factor from g to mg, wt=Sample weight (g). Data represented in mg of cyanidin-3-glucoside/g.

*H. Antioxidant Power for Reducing Ferric (FRAP)*

Potassium ferricyanide made a colored complex containing antioxidant properties to test the reducing power (Günsel, et al., 2019). A 0.1 mL extract sample of 3 mg/mL concentration was added to a tube containing 2.5 mL of 1% $K_3Fe(CN)_6$ and 2.5 mL of 0.2 M phosphate buffer (pH: 6.6). After 30 min at a temperature of 50°C, a 2.5 mL of 10% Trichloroacetic acid was added to the mixture and then centrifuged at 3000 rpm for 10 min. The supernatant was pipetted into a second tube containing 2.5 mL water and 0.5 mL of newly prepared 0.1% $FeCl_3$ concentration. The same approach was used to develop a standard calibration curve for quercetin (Fig. 3d). The absorbances were recorded at 700 nm.

*I. Metal Chelating Ability (MCA)*

The metal-chelating activity of blackberry extract was studied using the 1,10-phenanthroline technique (Akbaba, 2021) by filling the tube with 0.1 mL of sample solution (3 mg/mL). Then, 1.5 mL of water, 1 mL of 0.2% $FeCl_3$, 1 mL of 0.2% phenanthroline, and 1.4 mL of water were added. The tubes were vigorously shacked before incubation for 20 min in a dark place. The absorbance was determined at 510 nm. The equivalent iron II sulfate concentrations are given in mg $Fe^{2+}$/g DW (Fig. 3e).

*J. α-amylase Inhibition (AAI)*

A modified starch iodine approach was used (Ademiluyi and Oboh, 2013). A 0.25 mL of α-amylase was incubated with 0.25 mL (3 mg/mL) of plant extract for 15 min at 37°C. After the addition of 0.25 mL of starch solution, the mixture was reincubated for 30 min. A 0.1 mL of HCl (1.0 M) was added to halt the reaction. Adding 1.0 mL of iodine reagent to 3.0 mL of distilled water, the mixture was continuously vortexed. A spectrophotometer was used to quantify the absorbance at 580 nm. Individual blanks were prepared to change the background absorbance of the measurements. The controls were done the same way as the trials, where plant extract for 0.25 ml of distilled water was substituted. The commonly used drug for antidiabetic is acarbose, which was used as a positive control.

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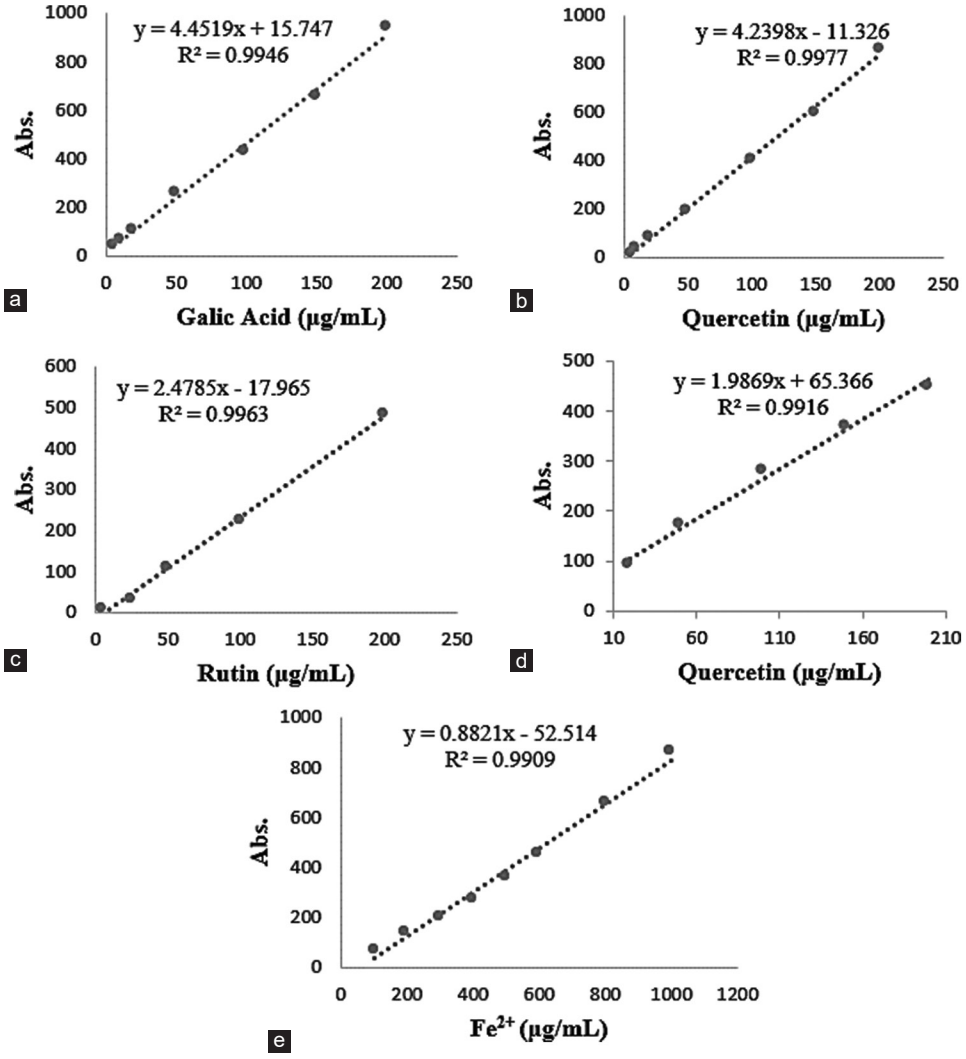


Fig. 3. Calibration curve of (a) gallic acid standard for the total phenolic content analysis, (b) quercetin standard for the total flavonoid content analysis, (c) rutin standard for the total flavonol content analysis, (d) quercetin standard for the ferric reducing antioxidant power analysis, and (e) Ferrous sulfate standard for the metal chelating ability analysis.

*K. Select a Plant Separation Solvent*

TLC confirmed the bioactive component separation from the methanolic extract. Many solvent systems were tested to choose the optimum one. The chromatogram was examined under UV light (254 nm and 365 nm) to see whether it could detect spots in an iodine chamber. The mobile phase employed was toluene-ethyl acetate-formic acid. Because of its higher $R_f$ value, chemical separation in column chromatography was achieved using toluene-ethyl acetate-formic acid (65:45:25).

*L. Chromatographic Separation in a Column*

Column chromatography is one of the best ways to separate phytocomponents. This study innovated an appropriate mobile solvent system. Therefore, the chromatography was done on an empty glass column filled with activated silica gel (70–230 mesh) and washed with the mobile phase. It was mixed with a solvent solution and put on the column (Fig. 4). TLC plates were applied to monitor the 85 collected fractions, and the relevant fractions were mixed, and then concentrated under low pressure. In the end, seven unique blackberry extract fractions were available.

*M. High-performance Liquid Chromatography (HPLC) Phenolic Components Analysis*

A Shimadzu HPLC equipped with a Shimadzu DGU-20A5 vacuum degasser and a Shimadzu 20 ADXR solvent pump was employed to determine the polyphenol concentrations. Separations were performed using a reversed-phase Clipeus C18 5 m column (250 mm × 4.6 mm). Detection was performed using a Shimadzu SPD-M20A photodiode array detector. Stock solutions for analytically pure polyphenol standards were prepared by dissolving 0.01 g of polyphenol in deionized clean water and adding 10 mL of MeOH and water in a (1:1) ratio. Essential stock solutions were prepared from the prepared stock solution for each polyphenol. HPLC-DAD method was used to identify the phenolic chemicals (Erkan, 2012). Gradient elution using solvent A: 4.5% of acetic acid solution and solvent B: acetonitrile as mobile phases at a flow rate of 1.0 mL/min and an injection volume of 20 µL

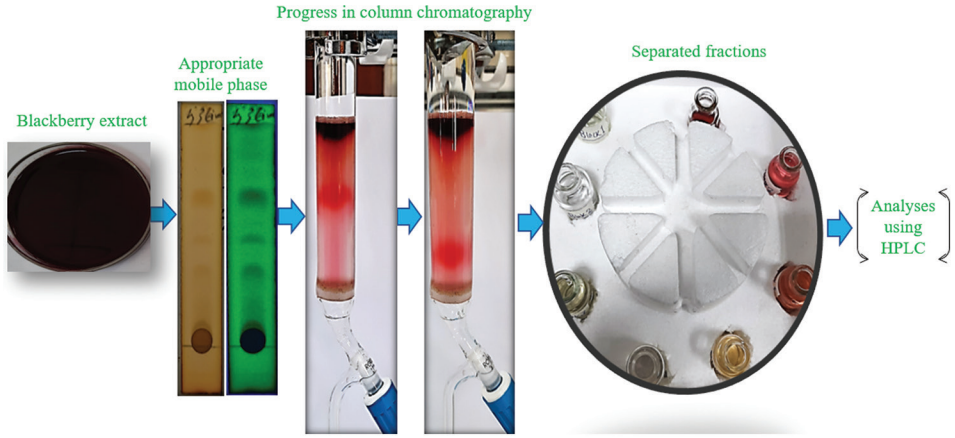


Fig. 4. Column chromatography produced seven fractions using a suitable mobile phase and thin-layer chromatography confirmation.

was used to detect the concentration. The diluted extract and the fractions were injected straight into the HPLC apparatus and screened using a photodiode array detector.

## III. Results and Discussion

### *A. Total Phenolic, Flavonoid, Flavonol, and Anthocyanin Contents*

Rubus fruticosus L.'s phenolic, flavonoid, flavonol, and anthocyanin content and the corresponding quantities are listed in Table I. The TPC of an extract was measured using the Folin–Ciocalteu method, which depends on transferring electrons from phenolic compounds to the Folin–Ciocalteu reagent in alkaline conditions. This method is a standard and straightforward one. TPC of 38.08 mg GAE/g dry extract was the most significant one. Many studies have been conducted to evaluate the correlation between phenolic content and antioxidant activity. Researchers observed a correlation between phenolic concentration and antioxidant activity, although others found no evidence (Ismail, Marjan, and Foong, 2004).

The human body's capacity to combat illnesses is aided by flavonoids, which are a subset of polyphenols (Rajanandh and Kavitha, 2010). Flavonoids are secondary metabolites that exhibit antioxidant activity based on the amount and position of free OH groups (Aryal, et al., 2019). This extract had 14.58 mg QE/g of dry extract of TFC, showing that it possessed the greatest TFC.

Many plant foods include flavonols, a class of flavonoids and polyphenols that critically prevent coronary heart disease and other age-related diseases such as dementia (Fang, Tang and Huang, 2013). Quercetin, the most abundant flavonol with antioxidant activity, has all the structural characteristics required for free radical scavenging (Kalita, et al., 2013). Blackberry extract had the greatest flavonols, with 6.95 mg RE/g dry extract of TF. The values of TPC and TFC in this study were more significant than the range previously reported by researchers. According to a study of the preceding literature, Zafra-Rojas, et al. reported a TPC of 40.16 mg GAE/g DW and a TAC of 3.65 mg cy-3-glu/g DW (Zafra-Rojas, et al., 2018). Diaconeasa et al. observed that the

TABLE I
Analyses of Blackberry Extract's Phytochemical Content

| Phytochemicals | Present quantity |
|---|---|
| TPC (mg GAE/g) | 38.08±0.19 |
| TFC (mg QE/g) | 14.58±0.83 |
| TF (mg RE/g) | 6.95±0.76 |
| TAC (mg cy-3-glu/kg) | 7.73±1.17 |

TPC: Total phenolic content, TFC: Total flavonoid content, TF: Total flavonol content, TAC: Total anthocyanin content, GAE: Gallic acid equivalent, QE: Quercetin equivalent, RE: Rutin equivalent, Results are reported as mean±SD of three determinations

TPC of blackberry methanol extract was 4.61 mg GAE/g DW and the TFC was 0.07 mg QE/g DW (Diaconeasa, et al., 2019). Koca and Karadeniz (2009) determined that the methanol extract of blackberry contained 3.26 mg of GAE/g and 1.59 mg of cy-3-glu/g of the dry weight of TPC and TAC (Koca and Karadeniz, 2009).

Phytochemicals called anthocyanins are naturally occurring pigments that give fruits, vegetables, and plants color. They stand head and shoulders above chlorophyll in readily observable plant pigments (Kong, et al., 2003). Glucosil residues and polar substituent groups (hydroxyl, carboxyl, and methoxyl) combined in anthocyanins, making them polar molecules in their own right (Navas, et al., 2012). *In vitro* and *in vivo* studies have shown anthocyanins' antiallergic, anti-inflammatory, antiviral, and antioxidant effects (Al-Sane, Povero, and Perata, 2011). Besides their various roles, they were reduced agents, hydrogen donors, and single oxygen quenchers. The presence of electron-donating and electron-withdrawing substituents in the ring structure and the amount and location of OH groups play an essential role in this property (Lapornik, Prošek, and Wondra, 2005). The most frequently used method for analyzing anthocyanin is the pH differential approach, because of its speed and simplicity. Blackberries had a 7.73 mg cy-3-glu/kg DW (Table II).

### *B. Antioxidant Power for Reducing Ferric*

One of the most used methods for evaluating antioxidant capability is the FRAP test. The ability of antioxidants to reduce $Fe^{3+}$ to $Fe^{2+}$ is the basis of the FRAP test's colorimetric technique. FRAP test directly reveals a substance's reducing capacity, which is essential for assessing whether a compound

is an efficient antioxidant (Rehakova, et al., 2014). In this study, a compound's potency as a reducing agent was evaluated by reducing Fe $[(CN)_6]^{3+}$ to Fe $[(CN)_6]^{2+}$. The complex formation results in an increase in absorbance, which shows that the reduction capacity has improved. Blackberry methanol extract had 20.53 mg $FeSO_4$/g DW (Table II). The antioxidant activity of blackberries has been reported in earlier studies to be typically lower than the value observed in this research. In the previous work, Zafra-Rojas, et al. discovered a FRAP of 12511.44 $\mu$mol Fe (II)/100 g DW (Zafra-Rojas, et al., 2018). Koca and Karadeniz (2009) previously determined FRAP equals 59.36 $\mu$mol Fe (II)/g DW (Koca and Karadeniz, 2009).

### *C. Capacity for Chelating Metal Ions*

Oxygen transport and respiration depend on iron, which acts as a cofactor for several iron-metallic enzymes. Fenton reactions, which are the breakdown of hydrogen or lipid peroxide to create highly reactive and physiologically hazardous hydroxyl and lipid peroxyl radicals catalyzed by transition metals, may cause the creation of reactive oxygen species if there is an excess of free iron (Islam, Yu and Xu, 2016).

The iron-chelating agent 1,10-phenanthroline is assumed to chelate primarily $Fe^{2+}$ as [Fe (phenanthroline)]$^{2+}$. $Fe^{2+}$-phenanthroline combination absorbs visible wavelength light at 510 nm, and the measurement is directly proportional to the sample's $Fe^{2+}$ level in the pH range of 2.5 to 8.0 (Zohlen, 2000). Antioxidants are oxidized when they are exposed to $FeCl_3$. Antioxidants undergo oxidation upon exposure to FeCl3, causing a reduction of Fe (III) to Fe (II). The Fe-phenanthroline generated matches a sample's antioxidant content (Yefrida, et al., 2018). The data show that blackberry extract can chelate metals at 182.12 mg $Fe^{2+}$/g DW (Table II). The blackberry methanol extract was shown to have FRAP of 955.4 $\mu$M $Fe^{2+}$/g DW and MCA of 437 $\mu$g/mL DW by Bhagat and Thusoo (2015).

TABLE II
Blackberry Extract ferric Reduction Antioxidant Power, Metal Chelating Capability, and AAI

| Antioxidants | Present quantity |
|---|---|
| FRAP (mg $FeSO_4$/g DW) | 20.53±0.47 |
| MCA (mg $Fe^{2+}$/g DW) | 182.12±0.26 |
| AAI% | 76.01±1.57 |

Results are reported as mean±SD of three determinations. DW: Dry weight, FRAP: Ferric reducing antioxidant power, MCA: Metal chelating ability, AAI: $\alpha$-amylase Inhibition

### *D. AAI Assay*

Antidiabetic drugs might be identified by investigating natural extracts from readily available traditional medicinal plants. Several plants have been showed to inhibit $\alpha$-amylase, making them potentially effective in treating diabetic patients (Rahimzadeh, et al., 2014). The human body needs to have an enzyme called $\alpha$-amylase that breaks down starch into simpler sugars (Nickavar and Yousefian, 2011). AAI slows the hydrolysis of $\alpha$-1,4-glycosidic bonds in carbohydrates (such as starch and maltose), resulting in decreased glucose absorption into the circulation (Anigboro, et al., 2021).

Inhibitory effects of blackberry extract on $\alpha$-amylase have been shown in inhibition studies. The amounts of plant extract in test samples were compared to those in control samples in all trials. Acarbose was used as a positive control. A plant extract's $\alpha$-amylase inhibitory activity was investigated using a quantitative starch-iodine method. The amylase inhibition effect on a plant extract is shown in Table II. Table III compares the phytochemical profile and antioxidant activity of the Kurdistan region to those of other regions.

### *E. HPLC Analysis of the Extract and Fractions*

A total of 85 fractions were collected using glass column chromatography. Using comparable thin-layer chromatograms, they were combined into seven separate fractions. Complex natural chemicals make it very difficult to investigate the structure of active plant components and evaluate their antioxidant and biological capabilities. High-resolution chromatographic technologies, such as HPLC, identified and characterized some of the bioactive compounds discovered in the extract and its fractions. (Gómez-Caravaca, et al., 2006). A fruit extract and seven blackberry fractions (F1-F7) were tested. As shown in Table IV, the fruit methanolic extract lacked significant phenolic components, while fraction two contained two different compounds and Fractions 4 and 5 included just one significant component. Other phenolics were detected; however, they could not be identified because of insufficient criteria.

TABLE III
Comparison of the Phytochemical Profiles of Blackberries from Kurdistan and Other Regions

| Phytochemicals | Kurdistan region | Other regions | References |
|---|---|---|---|
| TPC (mg/g) DW | 38.08±0.19 | 40.16 | Zafra-Rojas, et al., 2018 |
| | | 4.61 | Diaconeasa, et al., 2019 |
| | | 3.26 | Koca and Karadeniz, 2009 |
| TFC (mg/g) DW | 14.58±0.83 | 0.07 | Diaconeasa, et al., 2019 |
| TF (mg/g) DW | 6.95±0.76 | | |
| TAC (mg cy-3-glu/kg) DW | 7.73±1.17 | 3.65 | Zafra-Rojas, et al., 2018 |
| | | 1.59 | Koca and Karadeniz, 2009 |
| FRAP (mg/g) DW | 20.53±0.47 | 6.98 | Zafra-Rojas, et al., 2018 |
| | | 0.03 | Koca and Karadeniz, 2009 |
| | | 0.053 | Bhagat and Thusoo, 2015 |
| MCA (mg/g) DW | 182.12±0.26 | 0.437 | Bhagat and Thusoo, 2015 |

DW: Dry weight, TPC: Total phenolic content, TFC: Total flavonoid content, TF: Total flavonol content, TAC: Total anthocyanin content, FRAP: Ferric reducing antioxidant power, MCA: Metal chelating ability

TABLE IV
QUANTITIES OF PHENOLIC COMPOUNDS (MG/KG DM) IN VARIOUS BLACKBERRY FRACTIONS AND FRUIT EXTRACT

| Phenolic compounds | Extract | F1 | F2 | F3 | F4 | F5 | F6 | F7 |
|---|---|---|---|---|---|---|---|---|
| Chlorogenic acid | n.d. | n.d. | 0.084 | n.d. | n.d. | n.d. | n.d. | n.d. |
| Catechin | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| p-Coumaric acid | n.d. | n.d. | 0.961 | n.d. | n.d. | n.d. | n.d. | n.d. |
| Epicatechin | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Rutin | n.d. | n.d. | n.d. | n.d. | 0.404 | 3.134 | n.d. | n.d. |
| Resveratrol | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Naringin | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

n.d.: Not detected, DW: Dry weight

## IV. Conclusion

This research gives a complete analysis of the antioxidant potency of a blackberry fruit concentrate, fractionated by column chromatography. The extract was found to contain anthocyanin, flavonoids, flavonols, and polyphenolics, all of which have antioxidant properties. The high level of phenolic compounds, which have strong antioxidant and free radical scavenging properties, makes up around twice the amount of phenolic content compared to the total flavonoid content. Testing using FRAP and MCA revealed that this plant has a significant capacity for scavenging free radicals. The extract tested for its $\alpha$-amylase inhibitory capacity exhibited the most substantial inhibition. Related to validation parameters, standard deviations were given in the Tables of the results got. Linearity ranges were given on the calibration graphs. The limit of quantification (LOQ) values was found from the calibration graphs to be 5.0 µg/mL for TPC, TFC, and TF. For FRAP and MCA analyses, LOQs were determined as 20 and 100 µg/mL, respectively. The LODs values are one-third of the LOQ values given. For accuracy, the recovery tests were examined and it was found that those values are >90%. For the first time, column chromatography isolated seven different blackberry extract fractions. As part of the HPLC investigation, certain phenolic compounds, including p-coumaric acid, chlorogenic acid, and rutin, were discovered in fractions. For example, methanolic blackberry extract did not find any phenolic compounds similar to F1, F3, F6, and F7. While F4 and F5 had only rutin, F2 had p-coumaric acid plus chlorogenic acid. This study's discoveries could help identify the particular molecule that is causing the increased antioxidant activity in food.

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