Italian Journal of Food Science, 2025; 37 (1): 366–383

Wild cherry *Prunus microcarpa*: its phytochemical, antioxidant, enzyme inhibitory, anti-inflammatory, and acute toxicity approaches

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Academic Editor: Prof. Ana Sanches-Silva – University of Coimbra, Portugal

Received: 24 October 2024; Accepted: 17 December 2024; Published: 7 January 2025

ORIGINAL ARTICLE

Abstract

Therapeutic plants are potentially a great renewable biomolecule source with fewer downsides than synthetic chemicals. *Prunus microcarpa* Boiss. is a wild fruity plant prepared by hydro-distillation and consumed as a traditional remedy for many health defects. The study investigates the phytoconstituents, antioxidant, anti-inflammatory, and acute toxicity effects of methanolic extracts of *P. microcarpa* fruits and stems plus pedicles (MEPMF and MEPMSP). The total phenolics, flavonoids, anthocyanins, and tannins were spectrophotometrically estimated. The enzyme inhibitory action (against α-amylase and α-glucosidase), antioxidant potentials (using five different assays), and anti-inflammatory potentials (using the egg albumin denaturation procedure) were determined. Phytochemical profiling revealed increased total phenolics (76.34 and 121.45 mg gallic acid equivalent/g extract), flavonoids (210.30 and 18.93 mg GAE/g extract), anthocyanin (176.23 and 149.34 mg cyanidin 3-glucoside equivalent/g extract), and tannins (112.39 and 64.87 mg GAE/g extract) in MEPMF and MEPMSP, respectively. MEPMF had higher antioxidant potentials (130.83 and 117.12 mg TEs/g^{-1} extract in ABTS and DPPH assays, respectively) and higher reducing power actions (413.40, 141.23, and 97.62 mg TEs/g^{-1} extract in phosphomolybdenum, CUPRAC, and FRAP assays, respectively) than that of MEPMSP. MEPMF showed better inhibitory actions against α-amylase (IC_{50}; 6.70 mg/mL), α-glucosidase (IC_{50}; 5.72 mg/mL), and protein denaturation (IC_{50}; 53.78 mg/mL) than that (IC_{50}; 7.53, 6.28, and 84.42 mg/mL, respectively) of MEPMSP. According to biochemical evaluation, both extracts (2 and 5 g/kg) showed nontoxic effects in a two-week animal trial. In this first detailed record on *P. microcarpa,* the species exhibited numerous biological potentials attributed to its rich phytoconstituents, which also backed up its ethnomedicinal use.

Keywords: *Prunus Microcarpa*, phytochemical, antioxidant, enzyme inhibitory, acute toxicity

Introduction

Traditional medicine has a growing interest in health care in large parts of the world even if it is not accredited as an official treatment by the authorities. More than 80% of Asian and African citizens rely on natural products originating from plants to manage numerous health issues. The prevalence of medicinal plant use in pregnant women ranged from 19.2% to 90.2%. It was mainly used to reduce pain and inflammation (Bouqoufi *et al.*, 2023). Although interest in using natural products of plant origin was decreased for a while in the pharmaceutical industry, the incapability of obtaining combinatorial chemistry has revived scientific interest in natural resources for novel

 ISSN 1120-1770 online, DOI 10.15586/ijfs.v37i1.2856

discoveries. Indeed, such renewed interest is evidenced by the increased use of natural products as a main active ingredient for nearly 50% of approved pharmaceuticals (David *et al.*, 2015; de La Torre and Albericio, 2021). The ethnopharmacological investigation has recently become a valuable tool worldwide to shed light on species that may contain phytochemicals or molecules with potential use in pharmaceutical formulation, cosmetic, and dietary industries. Now, plants have been used as therapeutic agents more than ever by Western countries due to the downsides of chemical synthetics and the cost-effectiveness and safer after-effects of herbal medicine. Therefore, statistics show more than 4% per annum growth of industries associated with traditional therapies, even though the exact efficiency of plants and their phytochemicals is difficult to estimate (Atanasov *et al.*, 2021; Willis, 2017). The therapeutic action of herbal medicine and nonconventional plants is majorly linked to their diverse biomolecules, including polyphenols, flavonoids, anthocyanins, vitamins, and tannins (Elhouda-Mekhadmi *et al.*, 2024).

Natural products with potential bioactivities are extensively investigated for their therapeutic potential against numerous health issues, including obesity, cancer, and diabetes. Studies have shown the increased inhibitory potential of bioactive compounds against countless digestive enzymes, such as αamylase and α-glucosidase, as a regulatory mechanism for managing obesity and type 2 diabetes (Huang *et al.,* 2021). Moreover, research findings encourage food production enriched with bioactive molecules to generate better health efficiency. Some active ingredients need more time to interact with the cellular molecules and take more time to exert their antioxidant actions. Meanwhile, the synergistic use of diverse biomolecules results in much greater antioxidant actions at a much faster pace due to the interaction between such antioxidants (Newman and Cragg, 2020; Villalobos *et al.*, 2024). For example, toto peach fruits (*prunus persica* L.) are characterized by increased polyphenolic contents, flavonols, hydroxcinnamic acid, and anthocyanins in parallel with their significant antioxidant potentials (Bento *et al.*, 2022). Moreover, chemoprotective actions of the polyphenolic-rich extracts of *Prunus persica* L. Batsch have been linked with a range of protective pathways such as free radical scavenging action, carcinogen modulation, and regulation of xenobiotic metabolizing enzymes (Canistro *et al.*, 2016). Therefore, finding new natural biomolecules with increased bioactivities in plant species helps in designing better treatment regimens by using such active compounds in conventional pharmaceutical formulations. Numerous bioactive compounds from different plant species have shown efficient antioxidant and enzyme inhibitory potentials in different trials conducted worldwide (Achili *et al.*, 2020; Beddiar *et al.*, 2021; Trifan *et al.*, 2022). In contrast, some natural antioxidants belonging to phenolics have had some disappointing outcomes; for example, resveratrol improved the survival rate among mice ingested with a high-calorie diet, which was mainly explained by its provoked action on calorie-restriction metabolism unrelated to its antioxidant modulatory mechanisms (Canistro *et al.*, 2015). Coenzyme Q supplementation did not affect life longevity or antioxidant defense mechanisms in an animal model, while animals fed a diet without any antioxidant addition had extended life spans (Sohal *et al.*, 2006).

The ongoing argument could be explained by the fact that natural antioxidants during ROS elimination could be themselves modified into pro-oxidant by-products (semiquinone and quinone-like) when suitable co-antioxidant is absent and their radical scavenging potentials could be compromised by the pro-oxidant nature. Scientists declared that life span longevity is best achieved when a natural antioxidant acts by mimicking or provoking the SOD enzyme, an enzyme partitioning the superoxide anion without being modified chemically by the antioxidants (Vivarelli *et al.*, 2016).

Natural products are strong inflammation inhibitors by reducing inflammatory cytokine generation, thereby lowering protein denaturation and cellular alterations (Gomathi *et al.,* 2024). As one of the highest reported causes of inflammation, protein denaturation may result from injurious agents or infections that can lead to cellular dysfunctionalities eventually initiating numerous inflammation-related diseases. Thus, it is considered a valuable inflammatory indicator. In the past decades, numerous plants and their active ingredients have been reported as an efficient inhibitory of protein denaturation to an extent (Jabbar *et al.*, 2021; Thida *et al.*, 2024).

Since its first recognition by Guest and Townsend (1966), the Iraqi flora has been introduced with numerous *prunus species*, belonging to the subfamily Prunoideae, subdivided according to reproductive properties and morphological vegetation into subgenera Amygdalus, Padus, *Cerasus*, *Laurocerasus*, and *Prunus* (Guest and Townsend, 1966; Shan *et al.*, 2019). The subgenus amygdalin has been the major dietary source for honey bees, especially during winter and spring. Reduced genetic variability within the genus Prunus and its wild genetics make this plant genus a valuable source for numerous breeding potentials. As one of the wild prunus species, *prunus microcarpa* grows on dry calcareous hills and rocky mountains (up to 1000 m high) with increased resistant potential against the coldness of winter and dryness of summer (Khadivi *et al.*, 2022). Its potential has been linked to its nature as dwarfing rootstocks that generate precocious bearings or could be utilized as valuable diverse germplasm sources

(Khadivi *et al.*, 2022). Therefore, as a wild fruity plant, *P. microcarpa Boiss*. can be an ideal choice for investigation. In today's world, the majority of natural forests of these species have been demolished, and their population decline because of uncontrolled human behaviors as well as animal stress has led to genetic erosion despite their great potential to cope with environmental changes (Nuri Nas *et al.,* 2011). As a traditional medicinal plant, *P. microcarpa* has been processed using the hydro-distillation method and served as therapeutic tea (balaluk tea) for several health problems, including acute inflammatory thorax, common cold, kidney stones, and hair loss (Ahmed, 2016; Abdulrahman and Shahbaz, 2020; Khadivi *et al.*, 2022). The Prunus plant has been identified with numerous biological potentials, including antioxidant (Fu *et al.*, 2020), anti-inflammatory (Nunes *et al.*, 2022), anti-obesity (Song *et al.*, 2020), anti-diabetic (Kumar *et al.*, 2021), and anti-cancer (Kumar *et al.*, 2021) potentials that were mainly linked with its phytochemical compounds belonging to organic classes of phenolics, flavonoids, anthocyanins, tannins, and terpenoids (Telichowska, Kobus-Cisowska, and Szulc, 2020).

Thus, the present study attempted to shed light on the biological potentials of a Kurdish traditional medicinal plant, providing scientific data in conjunction with its traditional use, and to provoke further investigation. Our research identifies and quantifies the phytoconstituents of *P. microcarpa* to correlate with its determined antioxidant and enzyme inhibitory potentials against α-amylase and α-glucosidase enzymes. The acute toxicity effects were also evaluated using animal models to determine the safety dosage of this wild fruity species.

Methods

Plant collection and identification

The *P. microcarpa* fruits, stems, and pedicles were collected on slop hills of Warte River, Erbil, Iraq during the spring of 2024 (GPS position: 36.602006″N, 44.758786″E) (Figure 1). The plant species was authenticated by Dr. Abdullah Sardar, and the voucher number (745) was provided by Education College, Salahaddin University. The plant was washed with water and dried at 20–25°C in the shade. The dried plant is grounded and sieved to form a homogenous powder and stored in a tight sealed container for later use.

Preparation of methanol extracts

The prepared powders of *P. microcarpa* fruits and stems+pedicles (100 g) were separately macerated with absolute methanol (3×100 mL) using microwave-assisted extraction method (Panasonic P90N28AP-S3) at 800 W/5 min applying 20-s intervals of an irradiation cycle. The mixture solution was filtered and the solvent was separated under vacuum at 35°C using a rotary evaporator (P/N573-01300-00, Heidolph, Schwabach, Germany). The extraction was repeated three times, and plant yields of 39.42 g/100 g and 23.54 g/100 g were determined for MEPMF and MEPMSP, respectively. The obtained extracts were tight-sealed in dark vials to prevent contamination (Vinatoru *et al.*, 2017).

Ethical Approval

The present animal experiment was conducted in compliance with **ARRIVE** guidelines (Percie du Sert *et al.*, 2020). The animal handling protocols were approved by the ethical unit of Erbil Polytechnic University.

Phytochemical analysis

Qualitative measurements for phytochemical contents of *P. microcarpa* fruits and stems are conducted to ascertain the availability of carbohydrates, proteins, phenols, anthocyanins, saponins, alkaloids, quinones, tannins, flavonoids, phytosterols, terpenoid and cardiac glycosides following the basics of Harborne assays (Asiri *et al.*, 2024; Nortjie *et al.*, 2022). The quantitative evaluation of chemical contents followed previously published methodologies. The total phenolics and total flavonoids were determined using Folin–Ciocalteau's and the aluminum chloride ($AlCl_3$), respectively, with gallic acid as a standard. The total anthocyanin was estimated using the pH differential technique, which relies on the chemical structure alteration of anthocyanin and absorbance screening at pH 1.0 and 4.5 (Le *et al.*, 2019).

Total phenolic

Plant extracts at different intensities were poured into 150 µL of Folin–Ciocalteu phenol reagent (1 N) and were mixed well using a vortex. After incubation of the mixture at 37°C for 5 min, sodium carbonate solution (7.5%, 50 µL) was added. The reaction mixture was moved into an incubator for another 5 min at room temperature, and the absorbance was read at 725 nm (de Lima *et al.*, 2024). The data are shown as milligrams of gallic acid equivalent/g of extract.

Total flavonoid

The prepared different plant extract concentrations (1 mL) were mixed with aluminum chloride (2 mL). In addition, sodium acetate (6 mL) was added before the incubation process for 15 min at room temperature. The data are shown as milligram of gallic acid equivalent/g of

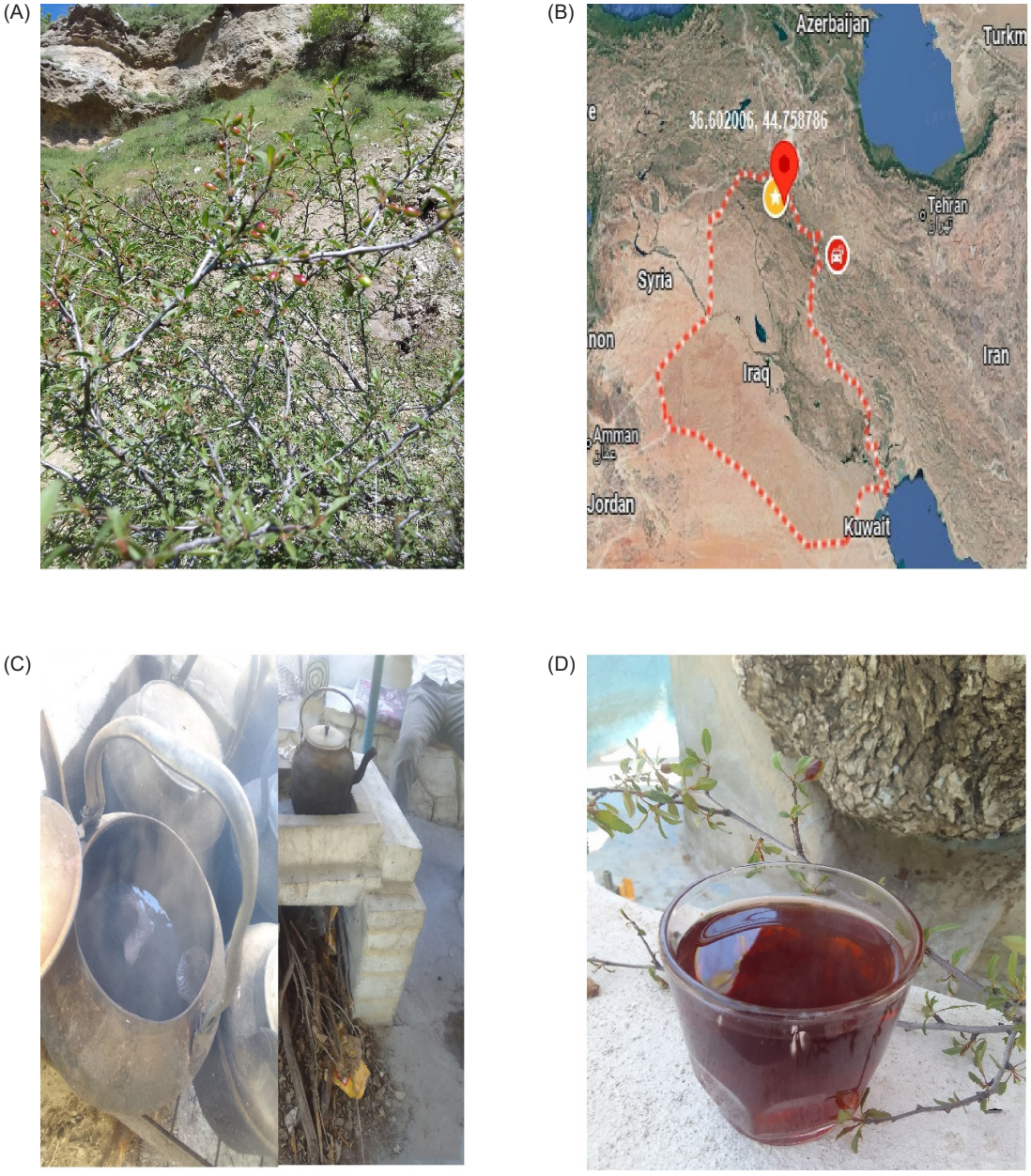

Figure 1. Aerial parts of *Prunus microcarpa* (A) collected from the Slop Hill of Warte River, Erbil, Iraq (B). The aerial parts of the plant were cooked (C) and served as tea (D) in the nearby roadside eatery (Photo taken by Ahmed A.j. Jabbar).

extract after reading absorbance at 415 nm (Erenler *et al.*, 2023).

Total anthocyanin content

An amount of 50 µL of different plant extract concentrations was poured into a solution of 75 µL of 1% vanillin, methanol, and 75 µL of 10% sulfuric acid. After incubating the mixture for 15 min at 30°C, the absorbance was taken at 500 nm. The results are expressed as milligram of cyanidin 3-glucoside equivalent/g extract (Hagos *et al.*, 2023).

Tannins

Tannin contents in MEPMF and MEPMSP were found following previously published protocols (Fadda and Mulas, 2010). An amount of diluted MEPMF and MEPMSP separately were mixed with 4 mL of the vanillin solution (constituted from 1% vanillin and 70% sulfuric acid) and 2 mL of ethanol. After incubating the reactive mixture at 37°C for 30 min, the absorbance was taken at 500 nm. The results are shown as milligram of gallic acid equivalent/g extract using the calibration curve (R2 = 0.99, 0.05–0.5 mg/mL of reference).

Antioxidant estimation

Total antioxidant estimation by phosphomolybdenum procedure

The total antioxidant potentials were analyzed using phosphomolybdenum as detailed by previous scientists (Zengin *et al.*, 2015). The sample solution (0.2 mL) was transferred into 2 mL of mixed reagent solution of 0.6 M sulfuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate. The samples were placed in an incubator (90 minutes at 95°C) before taking the absorbance (695 nm). The calculated total antioxidants were presented as equivalents of Trolox.

Antioxidant evaluation

The antioxidant potentials of MEPMSP and MEPMF in 10 µg/mL were estimated using the DPPH and ABTS assays. The reducing power potentials were found using CUPRAC and FRAP assays. The results are expressed as milligram Trolox/g equivalents (TE) (Wu *et al.*, 2023). For all antioxidant assays, MEPMF and MEPMSP at different concentrations were prepared before incubating them with other chemicals. The results are presented as IC_{50} (mg/mL) of Trolox, butylated hydroxyanisole, and butylated hydroxytoluene equivalents.

Anti-diabetic and enzyme inhibitory

The enzyme inhibitory potentials of *P. microcarpa* fruits and stem extracts followed the standards of previous studies (Lankatillake *et al.*, 2021). The data results were presented as IC_{50} and estimated by calculating the results of the inhibition percentage of plant extracts as follows:

$$\% \text{ activity} = \frac{\text{Absorbance of extract}}{\text{absorbance of control}} \times 100$$

For α-amylase inhibition estimation, 100 µL of plant extract was poured into a solution of starch and 20 mM phosphate buffer. After incubating the mixture for 10 min at 25°C, an amount of α-amylase enzyme (100 µL of 0.5 mg/mL) was delivered, and they were placed in an incubator (10 min at 25°C). The hydrolytic reaction termination was possible via the delivery of a dinitrosalicylic acid reagent (200 µL). The mixture solution was placed in an incubator (5 min at 100°C). After cooling the solution to 37°C, an amount of 50 µL reaction mixture was poured into a 96-well microtiter plate. In addition, in each well, distilled water (200 µL) was delivered to the mixture for sample dilution, and 540 nm was set for reading the absorbed light.

For α-glucosidase inhibition estimation, 50 µL of both extracts were separately poured into a solution of 100 mM sodium phosphate buffer (pH of 6.9). To start a hydrolytic reaction, 50 µL of PNPG (5 mM) solution was mixed with the solution. After incubating the mixture at 37°C for 5 min, 100 µL of phosphate buffer (0.1 U/mL of α-glucosidase enzyme) was poured and incubated altogether for 30 min. The absorbance of the reactive mixture was read at 405 nm.

Anti-inflammatory activity

The anti-inflammatory potentials of MEPMF and MEPMSP were estimated using an egg albumin denaturation procedure. In brief, 2 mL of extract concentration of MEPMF and MEPMSP/reference drug Diclofenac was mixed with 0.2 mL of egg albumin and 2.8 mL of phosphate buffer. An amount of the reaction mixture (5 mL) was placed in an incubator (37°C for 15 min). After keeping the mixture in a water bath (10 min, 70°C) for protein denaturation, the samples were cooled at room temperature and they were examined at 660 nm optical density in a microplate reader. In addition, egg albumin (2 mL) was mixed with phosphate buffer saline (2.8 mL) and evaluated as a negative control. The plant extracts inhibitory action on protein denaturation was found as follows:

$$\% \text{ inhibition} = 100 - \frac{\text{Ab}_t}{\text{Ab}_c - 1}$$

where Ab_t and Ab_c are absorbance values of test and control samples, respectively. The IC_{50} (extract concentration for 50% of inhibition) was found using an inhibition percentage plot of control against treatment intensity (Samaraweera *et al.*, 2023).

Acute toxicity

The toxic effect of MEPMF and MEPMSP was evaluated in a 14-day acute toxicity trial using oral gavage as a delivery method to rats. Thirty healthy Sprague rats of both genders (weighing about 180–200 g and aged 7–8 weeks) were provided by the Animal House Unit, Cihan University-Erbil, and the protocol followed the standards set by international regulations for laboratory animals. For the adaptation purpose, rats were kept in stainless steel mesh wire cages (to avoid coprophagia) at normal room temperature (25–27°C) and relative humidity (50–55%) with the availability of food and water. Rats were divided equally into five cages (three males and three females), fasted overnight (12 h without food), and received different oral treatments. After supplementation, food was withheld for an additional 4 hr. (Figure 2).

After the two-week observational process, all rats received an overdose of anesthesia intraperitoneal

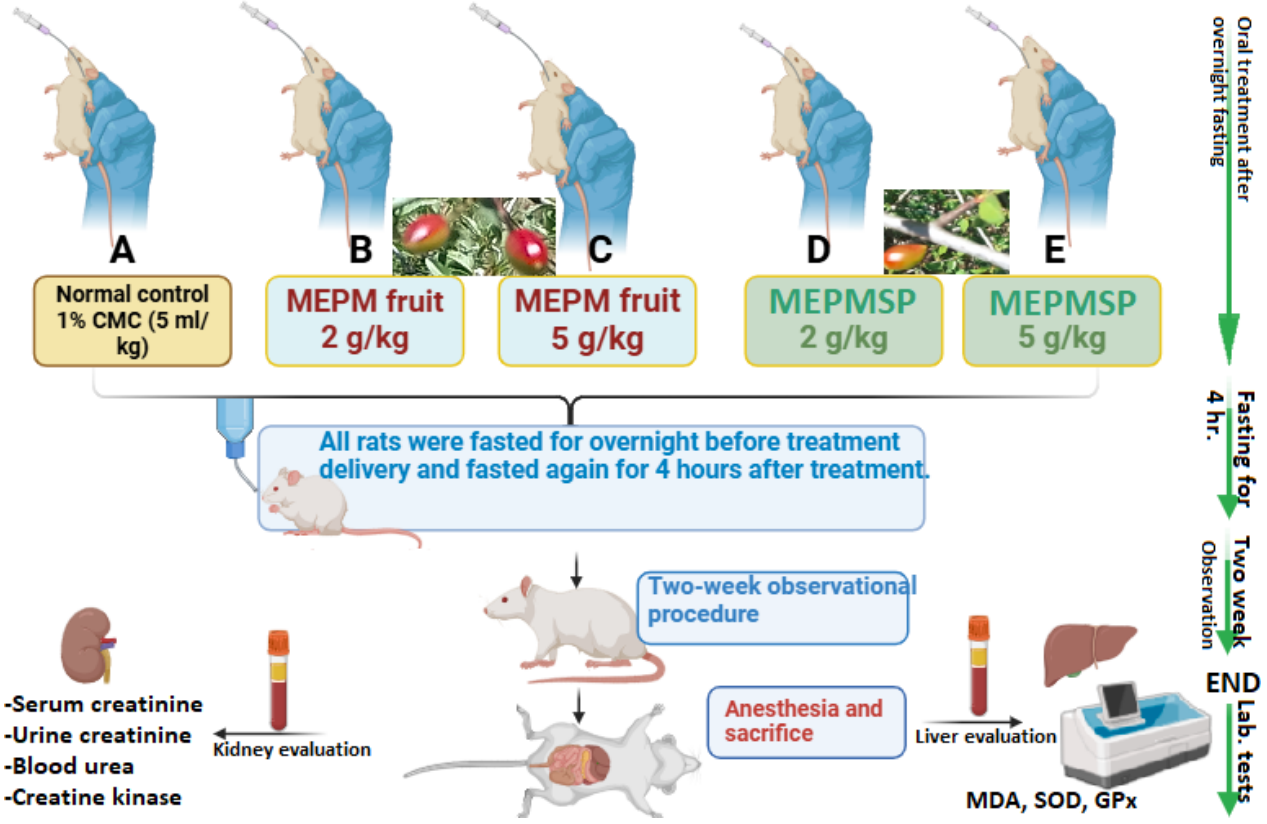

Figure 2. Experimental design of acute toxicity test for *P. microcarpa* extracts.

injection containing 30 mg/kg Ketamine and 3 mg/kg Xylazine and were sacrificed. The blood specimen from the cardiac puncture was obtained for biochemical estimations, including kidney and liver functional tests (Jabbar *et al.*, 2024; Alelign *et al.*, 2020).

Statistical analysis

The data were analyzed using the SPSS program, and values, including Tukey's test results, were presented as Mean ± STD. A one-way ANOVA was used to compare estimated biochemical parameters and a correlation coefficient (r) at $p \leq 0.05$ to assess the relationship between phytochemicals and detected biological potentials. The figures are generated using Graph-Pad Prism (Version 9.1).

Results and Discussion

Phytochemical profile

The extraction yield was higher in MEMPF (39.42 g/100) than that of MEPMSP (23.54 g/100 g). Similarly, the extraction yield of *Prunus laurocerasus* leaves was found in a range of 21.3–38.6 g ES/100 g. In contrast, its fruit extraction yield varied from 21.6 to 42.8 g ES/100 g using different extraction techniques (Classical, Microwave, Ultrasound, or Soxhlet-assisted extraction) (Karabegović *et al.*, 2014). A similar extraction yield of 41.30% was reported from Meghalayan cherry using an ultrasound extraction technique (Kashyap *et al.,* 2021). Therefore, the extraction yield must be kept in mind when selecting an extraction technique, and a high yield ensures an efficient evaluation procedure even though it does not guarantee an increased detection of phytochemicals (Nastić *et al.*, 2020). The qualitative evaluation of MEPMF and MEPMSP showed the presence of carbohydrates, proteins, phenols, terpenoids, anthocyanins, saponins, alkaloids, quinones, tannins, flavonoids, phytosterols, and cardiac glycosides, as presented in Table 1. The above-detected chemicals were confirmed by the previous phytochemical studies on different *prunus* fruit and stem extracts using spectrophotometric and chromatographic techniques (El-Beltagi *et al.*, 2019; Telichowska *et al.*, 2020).

Recently, polyphenols and flavonoid compounds have been extensively utilized in the therapeutic formulation of various acute and chronic health disorders, particularly those associated with ROS formation and oxidative

Table 1. Qualitative phytochemical profiles of MEPMF and MEPMSP.

Phytochemicals	MEPMF	MEPMSP
Carbohydrates	+++	+++
Proteins	++	+
Phenols	++	+
Alkaloids	++	+
Tannins	++	+
Anthocyanin	++	+
Flavonoids	++	++
Quinone	+	+
Phytosterols	++	+
Saponins	+	+
Cardiac glycosides	+	+
Terpenoids	+	++

Key: -, not present; +, trace; ++, moderate; +++, intense.

stress. Numerous clinical trials revealed that regular intake of a phenolic-rich diet can limit the risks of initiating several human diseases. Therefore, searching for safe sources of phenolic and flavonoid compounds has become the most attractive topic in scientific research investigations. In the present study, using colorimetric assays, the phytochemical quantification including total phenolic, flavonoid, tannins and anthocyanins in MEPMF and MEPMSP revealed significant variations between both plant extracts as shown in Figure 3. The total phenolic content was found via a calibration curve and presented as gallic acid equivalent. The methanolic extracts of *P. microcarpa* stem showed significantly (p<0.01) higher total phenolic contents (121.45 mg GA/g extract) than that (76.34 mg GAE/g extract) of its fruits. Total flavonoids were significantly (p<0.001) higher (210.30 mg GAE/g extract) in fruit extracts of *P. microcarpa* compared to that (18.93 mg GAE/g extract) of its stem extracts. The total anthocyanin content of MEPMF

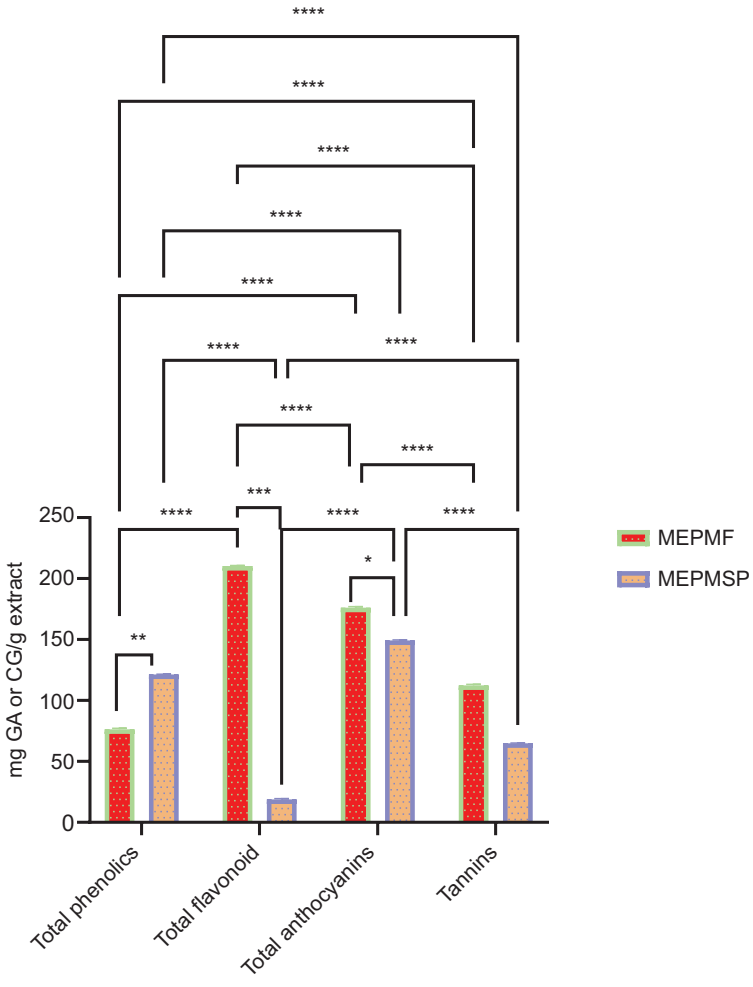

Figure 3. Phytochemical contents (total phenolics, flavonoids, tannins, and anthocyanins) in methanolic extracts of *P. microcarpa* fruits and stems (MEPMF and MEPMSP). Mean values of detected chemicals. The phytoconstituents of MEPF and MEPMSP were significantly varied at different levels indicated by asterisks. *, p < 0.05; **, p < 0.01; ***, p < 0.001, and ****, p < 0.0001.

extracts was significantly lower than the detected number of flavonoids. MEPMF showed significantly (p<0.05) higher (176.23 mg CGE/g extract) total anthocyanin contents than (149.34 mg CGE/g extract) in the MEPMSP. Similarly, bark extracts of *Prunus mahaleb* L. revealed higher total phenolic contents (5.11–131.70 mg GAE g^{-1}) compared to that (0.66–23.13 mg GAE g^{-1}) detected in fruit extracts, while, the flavonoid contents of fruit extracts (55.3–180.6 mg QE g^{-1}) was significantly higher than in the stem extracts (1.43–16.09 mg QE g^{-1}). Moreover, the total anthocyanin content of ten genotypes of *P. mahaleb* L. was found in 118.64 mg CY g^{-1} to 260.81 mg CY g^{-1} (Taghizadeh *et al.*, 2015). The present phytochemical profiling showed increased tannin contents (112.39 mg GAE/g extract) in the MEMPF than (64.87 mg GAE/g extract) in the MEPMSP. Similarly, increased tannin contents (20.42–163.4 mg catechin/g extract) were found in fruit extracts of *P. cerasus, P. avium, P. armeniaca, and Prunus domestica* (Çevik *et al.*, 2012; Song *et al.*, 2018). Accordingly, researchers have shown polyphenols, flavonols, organic acids, catechins, and tannins as major phytochemical content of fruit extracts obtained from *prunus padus* (Donno *et al.*, 2018). Fruit extracts of *p. padus* were revealed as a rich source of anthocyanin chemicals mainly cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-rutinoside, and cyanidin rhamnosyl hexoside (Mikulic-Petkovsek *et al.*, 2016). The variations in the phytochemical contents of different prunus fruits and stems in the literature data could be due to numerous factors, including, but not limited to, applied extraction techniques, different solvents, tissue variability of species, stages of fruit ripening, and the geographical and environmental factors (Kaseke *et al.*, 2020; Magangana *et al.*, 2020). The present primary detection of phytochemicals from extracts of *P. microcarpa* provides a ground point knowledge for future isolation of active ingredients underlining its bioactivities.

Antioxidant actions

Antioxidant potentials are widely relayed via estimation of the concentration required to the maximal effect (Gmax) or 50% of the optimal response (IC_{50}), which gives an idea about the lowest concentration to make a significant action (suppressing at least 50% of the reaction) that can be linked to other chemical and biological estimations (Zhang *et al.*, 2022). The plant extract screening for antioxidant potential is considered a valuable step toward the discovery of a novel phytochemical with therapeutic efficiency. The phosphomolybdenum, DPPH, and ABTS radicals are well-known scientifically approved free radicals used for evaluating the antioxidant properties of any herbal medicinal products (Vo *et al.*, 2023). Therefore, the present research evaluated the antioxidant and reducing power actions of two plant parts obtained from *P. microcarpa* using reference antioxidants such as Trolox, butylated hydroxytoluene, and butylated hydroxyanisole (Table 2).

The phosphomolybdenum scavenging reaction occurs by reducing molybdenum (VI) to molybdenum (V) and the reaction of antioxidants with a green phosphomolybdenum (Bibi Sadeer *et al.*, 2020). In the present study, MEPMF and MEPMSP showed a significant level of phosphomolybenum action, and the EC_{50} levels were lower (1.31±0.08) for MEPMF compared to that of MEPMSP (2.98±0.21). The 1 g extracts of MEPMF and MEPMSP were found to be equivalent to 413.40±4.82 and 285.73±3.70 mg of Trolox, respectively. According to the EC_{50} and TEs results, MEPMF has stronger phosphomolybdenum scavenging potentials compared to MEPMSP. These results are in parallel with the previous study reporting the phosphomolybdenum scavenging activity of *P. domestica* L. fruits within the range of 205.82±2.52 to 1554.15±5.73 µg AAE/mL. (Dhingra *et al.*, 2014). Moreover, the phosphomolybdenum scavenging action of *P. armeniaca, and* P. *domestica* L. were found to be within the range of 18.36±1.08–332.08±21.91 µM AAE/100g (Shan *et al.*, 2019).

As a stable, free radical, DPPH is considered an effective scavenging molecule that initiates a chemical reaction by replacing a nitrogen atom with a hydrogen atom for a reactive oxygen species (Jakubczyk *et al.*, 2021). In the present investigation, MEPMF revealed a stronger DPPH scavenging action than that of MEPMSP, as shown by a lower IC_{50} value of 2.31±0.22 mg/mL and a higher Trolox equivalent value (117.12±2.45 mg TEs/g) compared to MEPMSP (2.65±0.17 and 92.91±1.82 mg TEs/g extract) (Table 2). Similar data of IC_{50} and Trolox equivalent were found for ABTS scavenging actions. The IC_{50} and Trolox equivalent values were statistically varied between the two plant parts, with a lower IC_{50} (1.78±0.40mg/mL) and a high Trolox equivalent data (130.83±3.84 mg TEs/g extract) for MEPMF compared to that (2.16±0.55 mg/mL, 90.96±2.31 mg TEs/g extract) of MEPMSP. Thus, MEPMF was a stronger ABTS+ scavenger than MEMPS. The plant extracts with less IC_{50}, EC_{50}, and higher Trolox equivalent are labeled as a stronger antioxidant than those with high IC_{50}, EC_{50}, and a lower Trolox equivalent (Kirkan *et al.*, 2022). Accordingly, numerous researchers have shown the DPPH and ABTS scavenging actions of *Prunus* species with IC_{50} values within the range of 0.0729–4.19 mg/mL and 0.1896–2.89 mg/mL, respectively (Aliyazicioglu *et al.*, 2015; Dhingra *et al.*, 2014; Karakas *et al.*, 2019; Nowak *et al.*, 2020; Ozzengin *et al.*, 2023).

Plant extracts can exhibit antioxidant potentials capable of scavenging free radicals such as DPPH and

Table 2. Shows the antioxidant and reducing power action of fruits and stems of *Prunus microcarpa* based on different assays.

Assays	MEPMF	MEPMSP	Trolox	BHA	BHT
Phosphomolybdenum (EC_{50}:mg/ml)	1.31±0.08[b]	2.98±0.21[c]	0.14±0.06[a]	0.29±0.04[a]	0.37±0.03[a]
DPPH scavenging (IC_{50}:mg/ml)	2.31±0.22[c]	2.65±0.17[c]	0.26±0.02[a]	0.19±0.03[a]	1.01±0.1[b]
ABTS scavenging (IC_{50}:mg/ml)	1.78±0.40[b]	2.16±0.55[c]	0.33±0.04[a]	0.22±0.01[a]	0.29±0.02[a]
FRAP reducing (EC_{50}:mg/ml)	1.80±0.33[b]	2.30±0.10[c]	0.14±0.05[a]	0.10±0.03[a]	0.16±0.03[a]
CUPRAC reducing (EC_{50}:mg/ml)	1.94±0.28[b]	2.42±0.5[c]	0.29±0.10[a]	0.11±0.2[a]	0.18±0.01[a]
Phosphomolybdenum (mg TEs/g extracts)	413.40±4.82[b]	285.73±3.70[a]			
DPPH scavenging (mg TEs/g extracts)	117.12±2.45[b]	92.91±1.82[a]			
ABTS scavenging (mg TEs/g extracts)	130.83±3.84[b]	90.96±2.31[a]			
CUPRAC reducing (mg TEs/g extracts)	141.23±3.78[b]	97.85±2.64[a]			
FRAP reducing (mg TEs/g extracts)	97.62±2.63[b]	88.25±1.77[a]			

IC_{50}, inhibition concentration; EC_{50}, effective concentration; and different superscripts in a row represent the significance between plant parts and reference antioxidants at $p < 0.05$. Based on the applied assays of antioxidant and reducing power actions, there was a significant variation between MEPMF and MEPMSP potentials compared to that of Trolox using Tukey's test.

ABTS+ by an electron or hydrogen replacement. In contrast, FRAP reduction reaction occurs via an electron interaction associated with pH regulation (Wojtunik-Kulesza, 2020). The present study revealed better EC_{50} values of CUPRAC reduction (1.94 ± 0.28 mg/mL) and FRAP reduction (1.80 ± 0.33 mg/mL) for MEPMF than MEPMSP. According to both reducing power assays, MEPMF showed supremacy over MEPMSP. Similarly, reports have demonstrated significant reducing power potentials of *Prunus* species with values of 4.90–361.66 µmol TE/g and 19.69–149.13 μM Trolox/100g for CUPPRAC and FRAP reduction, respectively (Aliyazicioglu *et al.*, 2015; Ozzengin *et al.*, 2023; Shan *et al.*, 2019; Yüksekkaya *et al.*, 2021). Moreover, both plant parts had significantly higher EC_{50} values than standard antioxidants (such as Trolox and butylated hydroxytoluene). Overall, MEPMF exhibited better antioxidant potential than MEPMSP except for the DPPH scavenging action, which was non-significant ($p < 0.05$) between both plant parts. Moreover, the applied assays showed nonsignificant changes between standard antioxidants. The present antioxidant potentials of *P. microcarpa* parts could be correlated with its phytochemical profiles (polyphenols, anthocyanins, and flavonoids), which are repeatedly reported as strong antioxidant agents (Ademović *et al.*, 2017). The present spectrophotometric evaluation may provide an idea of the flavonoid + phenolic/antioxidant potentials in the prunus extracts. Overall, both plant samples exhibited good antioxidant actions, as estimated by radical scavenging, and metal-reducing, assays. However, interorgan differences were found. To complete this preliminary study, some *in vitro* (Pilařová *et al.*, 2024) and animal experiments are suggested.

Enzyme inhibitory effects

Plant extracts with increased phenolic and flavonoid contents could be a promising source for pharmaceutical formulation to treat numerous health disorders, particularly digestive disorders and diabetic conditions, because of their blood glucose–regulatory actions. The present enzyme inhibitory potentials are expressed as IC_{50}. Results indicated that compared to synthetic acarbose antioxidants (IC_{50}; 5.44 and 5.12 mg/mL for α-amylase and α-glucosidase, respectively), both plant extracts displayed remarkable suppression actions against the examined enzymes. The inhibitory action of MEPMF against α-amylase (IC_{50}; 6.70 mg/mL) was better than (IC_{50}; 7.53 mg/mL) of MEPMSP using the reference value of acarbose. The results also revealed the superiority of inhibitory activity (IC_{50}; 5.72 mg/mL) of MEPMF against α-glucosidase compared to that (IC_{50}; 6.28 mg/mL) of MEPMSP. These outcomes aligned with the literature data revealing a selective inhibitory capacity of phenolic-rich extracts against α-glucosidase rather than α-amylase (Bento *et al.*, 2018; Nowicka *et al.*, 2018). Moreover, the present data align with the previous findings correlating prunus fruit phytochemicals (flavonoids and anthocyanins) with better inhibitory actions on α-glucosidase than α-amylase enzymes (Nowicka *et al.*, 2016). Similarly, increased enzyme inhibitory potentials (IC_{50}; 10.25 ± 0.49 µg/mL) of 5 sweet cherry varieties have been linked with their polyphenolic and anthocyanin contents (Gonçalves *et al.*, 2017). Similarly, water and alcoholic extracts of *prunus spinosa L.* fruit exhibited better-suppressing action (IC_{50}; 0.22 and 0.08 mg/mL, respectively) on α-glucosidase than on the α-amylase (IC_{50}; 0.63±0.02 and 2.05 ± 0.05 mg/mL, respectively) (Marčetić *et al.*, 2022).

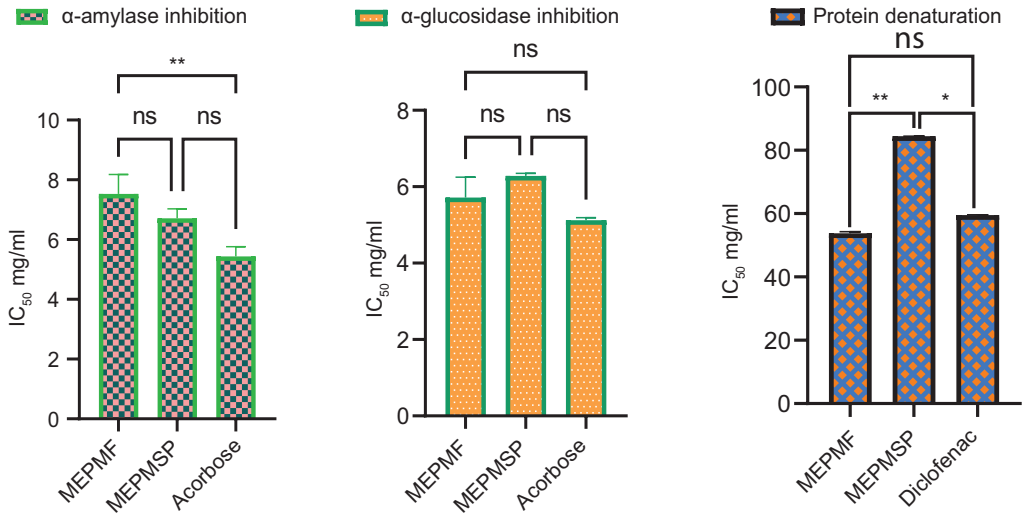

Figure 4. Inhibitory Effect of MEMPF and MEPMSP against α-amylase, α-glucosidase enzymes, and protein denaturation. The methanolic extracts of *P. microcarpa* fruits and stems plus pedicle (MEPMF and MEPMSP) showed differeninhibitory potentials on estimated enzymes and albumin denaturation. Asterisks indicate the significance level. *, p < 0.05; **, p < 0.01; ***, p < 0.001, and ****, p < 0.0001.

These results support MEPMF and MEPMSP as viable inhibitory bioactive molecules against glucose regulatory enzymes (α-amylase and α-glucosidase enzymes) that could serve as diabetic drug formulation (Figure 4).

Anti-inflammatory effects

The present study evaluates the anti-inflammatory potentials of MEPMF and MEPMSP via their inhibitory potentials against protein denaturation in egg albumin. Disruption of secondary and tertiary structures of proteins is considered an early stage of protein denaturation that may be initiated because of numerous stress factors altering their hydrophobic, hydrogen, electrostatic, and disulfide bonding. Protein denaturation is considered the highest reported causative factor of inflammation (Vaou *et al.*, 2021). As expected, the present results showed that the concentration of Diclofenac for 50% inhibition of albumin denaturation was found as (IC_{50}; 59.54 mg/mL). The interesting result was detecting efficient inhibitory potentials (IC_{50}; 53.78 mg/mL) of MEPMF against protein denaturation, which were comparable and non-significantly varied compared to the reference drug. MEPMSP showed moderate inhibitory potentials on protein denaturation, in which the concentration of MEPMSP for 50% inhibition of protein denaturation (IC_{50}) was 84.42 mg/mL, which was significantly varied (less efficient) compared to MEPMF and Diclofenac reference drug (Figure 4). Accordingly, researchers reported significant anti-inflammatory potentials of different fractions of *prunus* fruits and stems (*P. yedoensis*) in different *in vitro* and *in vivo* approaches, which were mainly linked with their polyphenolic and flavonoid contents (Kang *et al.*, 2015; Yun *et al.*, 2014). Similarly, methanolic extracts of fruits, stems, and leaves of *p. persica L.* exhibited significant anti-inflammatory actions on lipopolysaccharide-mediated inflammation glial cells that were mainly linked with their phenolic (chlorogenic and catechin) potentials in lowering transcription of numerous inflammatory mediators (cyclooxygenase2 and nitric oxide synthase) and cytokines (tumor necrosis factorα, interleukin6, and IL1β) as well as suppressing the NFκB and several mitogen-activated protein kinases pathways required for inflammation initiation (Seo *et al.*, 2020). The present outcomes could serve as a feedstock for further investigation on the anti-inflammatory potentials of this plant species in broader study trials.

Correlation between antioxidant activity, phytochemical content, and enzyme inhibitory actions

To elucidate the link, a Pearson correlation of the phytochemical contents, antioxidants, and enzyme inhibitory potentials was conducted. In MEPMF, the total phenolic, flavonoid, anthocyanin, and tannin contents significantly correlated with the antioxidant activities found in the CUPRAC assay (Table 3). Moreover, the phenolic content of MEPMF showed a significant positive correlation with the total antioxidant potentials detected in phosphomolybdenum and its inhibitory potentials on α-amylases and α-glucosidase actions.

The correlations detected between the bioactivity of *P. microcarpa* extracts and their chemical contents are

Table 3. The correlation coefficient (r) between the phytochemical contents, antioxidants, and enzyme-inhibitory actions of fruit extracts of P. microcarpa.

Assays and chemicals	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Phosphomolybdenum	1.000															
DPPH	0.398	1.000														
ABTS	–0.002	–0.141	1.000													
FRAP	0.090	0.566	–0.253	1.000												
CUPRAC	0.307	0.049	0.038	–0.376	1.000											
Phosphomolybdenum 1	0.436	0.494	0.217	0.625	–0.094	1.000										
DPPH Extracts	–0.646	–0.239	0.311	–0.347	0.061	–0.586	1.000									
ABTS Extracts	0.223	0.178	0.583	0.002	–0.043	0.284	0.342	1.000								
CUPRAC	0.004	–0.668	0.102	–0.394	–0.004	0.064	–0.343	–0.117	1.000							
FRAP Extracts	–0.385	–0.350	0.175	–0.418	0.381	–0.427	0.778	0.282	–0.133	1.000						
α-amylase inhibition	–0.039	0.051	0.100	0.309	–0.009	0.658	–0.283	–0.053	0.133	0.048	1.000					
α-glucosidase inhibition	0.172	–0.282	–0.296	–0.306	0.470	–0.396	0.126	–0.154	0.010	0.589	–0.141	1.000				
Total Phenolics	0.261	0.007	0.130	0.165	0.560	0.509	–0.270	0.116	0.379	0.124	0.361	0.242	1.000			
Total Flavonoid	–0.267	–0.511	0.142	–0.522	0.070	–0.095	0.234	0.136	0.517	0.594	0.366	0.374	0.201	1.000		
Total Anthocyanins	–0.135	–0.650	0.027	–0.040	–0.422	–0.203	0.133	0.101	0.262	0.310	0.035	0.413	–0.124	0.366	1.000	
Tannins	–0.066	–0.427	0.269	–0.321	–0.406	0.110	–0.282	–0.147	0.635	–0.180	0.252	–0.065	–0.083	0.564	0.337	1.000

A, phosphomolybdenum in EC50; B, DPPH in IC50; C, ABTS in Ic50; D, FRAP in Ec50; E, CUPRAC in EC50; F, phosphomolybdenum (mg TEs/g extracts); G, DPPHscavenging (mg TEs/g extracts); H, ABTS scavenging (mg TEs/g extracts); I, CUPRAC reducing (Ec50: mg/ml); J, FRAP reducing (mg TEs/g extracts); K, α-amylase inhibition ic50; L, α-glucosidase inhibition IC50; M, total phenolics; N, total flavonoid; O, total anthocyanins; P, tannins.

In the correlation Table 3, each cell was colored differently to indicate the strength of positive/negative correlation, in which intense green colors indicate perfect positive correlation (1) but intense red color indicates perfect negative correlation (-1). Meanwhile, the less intense color between these two shows a less-than-perfect correlation (i.e., Red is -1, 100% negative, while Green is 1, 100% positive).

Table 4. The correlation coefficient (r) between the phytochemical contents, antioxidants, and enzyme-inhibitory actions of stem extracts of *P. microcarpa*.

Assays and chemicals	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Phosphomolybdenum	1.000															
DPPH	–0.368	1.000														
ABTS	0.102	–0.110	1.000													
FRAP	–0.002	–0.022	0.115	1.000												
CUPRAC	0.062	–0.220	–0.230	–0.504	1.000											
Phosphomolybdenum	0.532	–0.738	0.088	–0.282	0.178	1.000										
DPPH Extracts	0.139	–0.184	–0.418	–0.467	0.192	0.530	1.000									
ABTS Extracts	–0.097	0.105	–0.080	–0.198	–0.309	0.202	0.281	1.000								
CUPRAC	–0.016	0.322	0.200	0.225	0.288	–0.584	–0.740	–0.531	1.000							
FRAP Extracts	–0.089	–0.357	–0.193	0.525	–0.058	0.170	–0.347	0.102	0.067	1.000						
α-amylase inhibition	–0.028	–0.133	–0.304	0.382	–0.231	–0.072	–0.207	0.249	–0.242	0.470	1.000					
α-glucosidase inhibition	–0.695	0.407	–0.619	–0.032	0.017	–0.413	0.314	0.197	–0.304	–0.029	0.349	1.000				
Total Phenolics	–0.282	–0.213	0.332	–0.436	0.274	0.324	–0.034	–0.248	–0.026	0.031	–0.409	–0.177	1.000			
Total Flavonoid	0.037	0.230	–0.163	0.544	–0.064	–0.488	–0.583	–0.700	0.576	0.261	0.279	0.014	–0.123	1.000		
Total Anthocyanins	–0.448	0.085	–0.541	–0.001	–0.093	–0.509	–0.073	–0.166	0.013	–0.022	0.277	0.509	–0.178	0.274	1.000	
Tannins	–0.024	0.83	–0.030	0.188	0.147	–0.078	–0.377	–0.001	0.557	0.546	–0.299	–0.276	0.139	0.191	–0.295	1.000

A, phosphomolybdenum in EC50; B, DPPH in IC50; C, ABTS in Ic50; D, FRAP in Ec50; E, CUPRAC in EC50; F, phosphomolybdenum (mg TEs/g extracts); G, DPPHscavenging (mg TEs/g extracts); H, ABTS scavenging (mg TEs/g extracts); I, CUPRAC reducing (Ec50: mg/ml); J, FRAP reducing (mg TEs/g extracts); K, α-amylase inhibition ic50; L, α-glucosidase inhibition IC50; M, total phenolics; N, total flavonoid; O, total anthocyanins; P, tannins.
In the correlation Table 3, each cell was colored differently to indicate the strength of positive/negative correlation, in which intense green colors indicate perfect positive correlation (1) but intense red color indicates perfect negative correlation (-1). Meanwhile, the less intense color between these two shows a less-than-perfect correlation (i.e., Red is -1, 100% negative, while Green is 1, 100% positive).

particularly noteworthy, as they can attribute some of the biologically active compounds. For instance, the antioxidant actions detected in the FRAP method were strictly correlated with the total flavonoid contents of MEPMF. The α-glucosidase enzyme inhibitory potentials of MEPMF had the strongest positive correlation with the total flavonoid and anthocyanin contents. While tannin phytochemical was majorly associated with antioxidant potentials of MEPMF detected in ABTS and CUPRAC assays and α-amylase inhibitory action. The total flavonoid content of MEPMF was strictly positively correlated with its tannin, anthocyanin, and phenolic contents, respectively (Table 3).

In MEPMSP, the total flavonoid and tannin contents had a strong (p<0.01) positive correlation with the reducing power actions detected in FRAP and CUPRAC assays while its phenolic content was positively associated with antioxidant actions recorded with ABTS, CUPRAC, and phosphomolybdenum procedures. The total flavonoid and anthocyanin content had a weak positive correlation in the methanolic extracts of *P. microcarpa* stems and pedicles (MEPMSP). The antioxidant actions found in the DPPH assay revealed a strong positive association compared to phosphomolybdenum's value. Moreover, the total anthocyanin was found to be intercorrelated strongly with the obtained α-glucosidase inhibitory potentials, but a weak correlation was observed with the detected α-amylase inhibitory potentials. Overall, the enzyme inhibitory potentials of MEPMSP were positively involved with estimated total flavonoid and anthocyanin contents. Regarding enzyme and antioxidant association, the enzyme inhibitory potentials of MEPMSP were positively correlated with its antioxidant actions found in DPPH, ABTS, and FRAP assays (Table 4). Consistent with the present results, these phytochemicals were reported as natural antioxidants in numerous detailed investigations (Filaferro *et al.*, 2022; Hamrouni *et al.*, 2023; Nardini, 2023; Paula Sales *et al.*, 2023). Moreover, a significant positive correlation between phytoconstituents and their anti-enzymatic and antioxidant potentials, particularly free radical quenching and reducing power actions, has been found in several studies conducted on members of the *Prunus* species (Amir *et al.*, 2022; Marčetić *et al.*, 2022; Pradhan *et al.*, 2022; Telichowska, Kobus-Cisowska, and Szulc *et al.*, 2020).

Acute toxicity effects

Toxicological evaluation of any wild plant is considered an initial precautionary step before considering it in repeated doses for longer periods. After a 2-week supplementation, the results revealed a lack of any toxic signs or physiological modifications in rats that consumed up to 5 g of MEPMF and MEMPS with no mortality or morbidity throughout the 2-week trial. Rats appeared without any physical changes in their fur and eye color, feed and water intake, behavior, and locomotion were comparable to normal controls (Table 5). The regular observation found no toxic signs and symptoms in rats such as salvation, tremors, convulsion, lethargy, breathing problems, depilation, or diarrhea. The serum profiling of plant extract-ingested rats showed a nonsignificant liver and kidney function alteration compared to the normal control rats (Table 5). The present results are found in alignment with published data demonstrating the safe consumption of prunus extracts (*P. domestica, P. africana,* and *P. armaniaca*) in doses ranging from 1–5 g/kg delivered to different animal models (Karani *et al.*, 2013; Kumar *et al.*, no date; Seniuk *et al.*, 2022).

Table 5. Effect of MEPMF and MEPMSP on serum and urine biochemical profiles.

Parameters	A	B	C	D	E
Weight (g)	255±10.3	271±21.5	278±23.7	248±14.8	271±21.5
Feed intake (g)	19.8±3.1	18.4±3.2	19.2±3.3	19.5±3.0	18.9±3.2
Water intake (ml)	25±4.2	31±4.3	34±3.5	28±6.3	29±3.3
Urine volume (ml)	11.2±3.2	11.9±3.7	12.9±4.4	11.3±3.1	11.8±4.3
Urine creatinine (mg/dl)	54.2±3.2	54.8±4.2	56.5±3.8	53.4±2.4	54.3±3.3
Serum creatinine (mg/dl)	0.8±0.03	1.1±0.08	0.9±0.04	0.7±0.03	1.1±0.08
Serum BUN (mg/dl)	48.8±3.1	51.2±3.4	52.2±3.5	49.1±3.3	51.3±3.9
SOD (U/mg)	169.5±5.8	137.4±5.9	154.5±4.9	163.5±5.9	136.8±5.8
MDA (nmol/mg)	2.4±0.9	2.6±0.7	2.5±0.7	2.5±0.6	3.1±0.9
GSH-Px (U/mg)	3.4±1.1	2.4±0.8	2.1±0.9	3.1±1.2	2.7±1.2
ck (U/l)	192.5±6.8	221.2±10.9	195.9±9.8	191.4±9.6	202.8±8.5

Group A rats received 10% tween 20 (5 mL/kg); groups B and C rats ingested 2 and 5 g/kg of MEMPF, respectively; group D and E rats ingested 2 and 5 g/kg of MEPMSP, respectively.

Conclusion

This study allowed for the determination of polyphenols, flavonoids, anthocyanins, and tannins as the most common chemical classes of wild cherry *P. microcarpa* extracts with a predominance of fruit extracts over stems except for the total phenolic content. Compared to reference groups, both plant part extracts exhibited remarkable antioxidant potentials and reducing power potentials according to the results of four different assays. The enzyme inhibitory potentials of MEPMF against α-amylase and α-glucosidase were remarkably higher than that of MEPMSP. Moreover, both extracts displayed noticeable inhibitory actions on albumin denaturation, indicating possible anti-inflammatory potentials. Overall, the outcomes shed light on *P. microcarpa* by-products as a renewable feedstock exhibiting several biological potentials without toxic effects based on *in vivo* acute toxicity experiments. The present study's limitations included the unavailability of essential kits and reagents, the small budget, and the inadequacy of laboratory instruments. Therefore, future investigations (e.g. identifying and isolating the main active ingredients and exploring pathways that underline their biological potentials) in broad cell-based or animal models would comprise a further step before considering it as a viable source for developing cosmeceuticals, nutraceuticals, and pharmaceuticals to manage oxidative stress and inflammatory-related health disorders.

Abbreviation

DPPH, 2,2-Diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonicacid;CUPRAC, Cupric ion reducing antioxidant capacity; FRAP, Ferric reducing antioxidant power; TEs, trolox; BHA, butylated hydroxyanisole, BHT, butylated hydroxytoluene; BUN, blood urea nitrogen; SOD, superoxide dismutase; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; CK, creatine kinase; PNGP, p-nitrophenyl-α-D-glucopyranoside.

Data Availability

Further details are available on request.

Authors' Contributions

A.A.J. and P.A.I. participated equally in conceptualization, structure and design, methodology, data analysis, resources and validation, software, and original draft writing.

Conflicts of Interest

None.

Funding

None.

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