Original Research

LC-ESI-QTOF-MS/MS characterization of phenolic compounds from *Prosopis farcta* (Banks & Sol.) J.F.Macbr. and their potential antioxidant activities

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Abstract: *Prosopis farcta* (Banks & Sol.) J.F.Macbr. is an emerging medicinal plant containing a diverse array of phytochemicals, including protein, fat, carbohydrate, fibre, alkaloids, fatty acids, glycosides, and polyphenols, with strong antioxidant potential. However, the screening and characterization of phenolic compounds in *P. farcta* is limited. This study is conducted to determine the polyphenol contents and their antioxidant activity in *P. farcta* leaves samples via liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS/MS) and high-performance liquid chromatography-photodiode array (HPLC-PDA). Total phenolic content (TPC), total flavonoid content (TFC), and total tannins content (TTC) were determined for polyphenol estimation. The antioxidant properties were measured by total antioxidant capacity (TAC), 2,2'-Diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). LC-ESI-QTOF-MS/MS was used to identify and characterize 47 phenolic compounds, which mainly included phenolic acids (13), flavonoids (28), other polyphenols (4), lignans (1), and stilbenes (1). According to HPLC-PDA quantification, chlorogenic acid (9.78 ± 2.15 mg/g dw) was the most abundant phenolic acid, while the main flavonoids included catechin (12.73 ± 1.29 mg/g dw) and kaempferol (7.93 ± 1.47 mg/g dw). The study demonstrated the significance of *P. farcta* as a rich source of phenolic compounds with antioxidant capacity that can be widely used in food, beverage, feed, and pharmaceutical applications.

Key words: Medicinal plants; polyphenols; phenolic compounds; *Prosopis farcta* (Banks & Sol.) J.F.Macbr.; antioxidant capacity; LC-ESI-QTOF-MS/MS; HPLC-PDA.

Introduction

Leguminous plants can be consumed in varieties of dishes and can be considered medicinal plants (1). The medicinal functions are mainly attributed to bioactive compounds, especially different kinds of polyphenols (2). Due to the polyphenolic contents, leguminous intake can reduce the risk of diabetes, metabolic syndrome, cardiovascular diseases, and cancers (3). Therefore, there is an increasing interest in studying the polyphenolic compounds of leguminous plants and their antioxidant capacity (4).

Prosopis genus is an underutilized legume plant that belongs to the *Leguminosae* family (1). *Prosopis* genus comprises 44 species, including *P. juliflora*, *P. farcta*, *P. velutina*, *P. glandulosa*, *P. laevigata*, *P. pallida*, *P. cineraria*, etc. (5). *Prosopis* is mainly distributed in arid, semi-arid, tropical, and subtropical countries, such as America, India, Argentina, Chile, Kenya, and Pakistan (6). The ecological value of *Prosopis* plants due to their resistance to heat, drought, salinity, and alkalinity, while

Prosopis can also promote nitrogen fixation to stabilize and improve the soil (7). The leaves of *Prosopis* plants are glabrous or puberulous and deciduous, with a length of 2-7 cm (2). The fruits are elongate, slender, 10-21 cm long, while the peel is brittle and thin (2). The seeds have a brown color and ovate shape (8). *Prosopis* plants can be consumed as beverages, flour, sweets, jams, bread, cakes, cookies, and syrup (3). The *Prosopis* flavor is brown and sweet, with a similar aroma to coffee, cocoa, coconut, or caramel (9). Besides, the *Prosopis* gum, which is exuded from the *Prosopis* tree's bark, can be used as an emulsifier, film-forming agent, foaming agent, tablet binder, and stabilizer (7).

The chemical compounds of *Prosopis* plants are comprised of protein, fat, carbohydrate, fibre, alkaloids, fatty acids, glycosides, sterols, free amino acids, minerals (phosphorus, calcium, and iron), and significant quantities of polyphenolic compounds (10). In previous studies, polyphenols in *Prosopis* plants mainly include flavonoids, tannin, anthocyanin, gallocatechin, coumaric acid, morin, rutin, catechin, gallic acid, naringenin,

and epicatechin (5). Polyphenols are important groups of secondary metabolites, consisting of one or more aromatic rings attached to hydroxyl groups (10). They have a strong antioxidant capacity that can scavenge free radicals, prevent the formation of free radicals and prevent lipid peroxidation via hydrogen atom transfer, electron transfer, and the chelation of the metal, (11). *Prosopis* plants have plenty of medicinal functions including antioxidant activity, antimicrobial, antihyperglycemic, analgesic, anticonvulsant, antihyperlipidemic, antidepressant, hypolipidemic, and antiatherosclerosis activities because of the presence of these polyphenolic compounds (12).

The antioxidant activity of polyphenols can be determined by various spectrophotometric-based methods based on different mechanisms, consisting of determination of total phenolic content, free radical scavenging methods, non-radical redox potential-based methods, and metal-chelating methods (13). Therefore, a set of assays, which includes total phenolic content (TPC), total flavonoid content (TFC), total tannin (TTC), total antioxidant capacity (TAC), 2,2'-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing of antioxidant power (FRAP), and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), will be used to measure the phenolic contents and antioxidant activity of *Prosopis* plants. The antioxidant activity might vary due to the samples and the types and characteristics of solvent extraction (14). To develop the extraction yields, water, ethanol, methanol, and acetone are acted as solvents to extract chemical compounds (14). However, after the extraction, the isolation and identification of phenolic compounds, such as anthocyanins, are difficult due to the structural diversity (15). Liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS/MS) is an innovative analytical tool that is more sensitive and accurate. It can be effectively used to identify and quantify both low and high molecular weight phenolic and non-phenolic compounds (16). Besides, a high-performance liquid chromatography-photodiode array (HPLC-PDA) will be used to separate, purify, and measure the individual phenolic compounds and other bioactive compounds (17).

The present research aims to extract polyphenolic compounds from the *Prosopis farcta* (Banks & Sol.) J.F.Macbr. sample, measure the phenolic contents through TPC, TFC, and TTC, and determine the antioxidant capacity by TAC, DPPH, FRAP, and ABTS. Further, phenolic compounds will be isolated, identified, characterized by LC-ESI-QTOF-MS/MS, and quantified through HPLC-PDA.

Materials and Methods

Chemicals

In this study, most of the chemicals, reagents, and standards used for extraction and identification were bought from Sigma-Aldrich (Castle Hill, NSW, Australia). Besides, the chemicals for estimating polyphenols and antioxidant potential, including Folin-Ciocalteu's phenol reagent, gallic acid, L-ascorbic acid, vanillin, hexahydrate aluminum chloride, sodium phosphate, iron(III) chloride hexahydrate (Fe[III]Cl3.6H2O), hy-

drated sodium acetate, hydrochloric acid, ammonium molybdate, quercetin, catechin, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6tripyridyl-s-triazine (TPTZ), and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from the Sigma-Aldrich (Castle Hill, NSW, Australia). HPLC standards, which included gallic acid, protocatechuic acid, caftaric acid, p-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, coumaric acid, ferulic acid, sinapinic acid, catechin, epicatechin gallate, quercetin-3-galactoside, quercetin-3-glucuronide, quercetin-3-glucoside, quercetin, diosmin, kaempferol and kaempferol-3-glucoside were also bought from Sigma-Aldrich (Castle Hill, NSW, Australia) and aimed to quantification. Sulfuric acid (H_2SO_4) with 98% purity was bought from RCI Labscan (Rongmuang, Thailand), while Sodium carbonate anhydrous was purchased from Chem-Supply Pty Ltd. (Adelaide, SA, Australia). Methanol, ethanol, acetonitrile, formic acid, and glacial acetic acid were the HPLC and LC-MS grade reagents that were bought from Thermo Fisher Scientific Inc (Scoresby, VIC, AU). 96 well-plates, which were used to perform plenty of in vitro bioactivities and antioxidant assays, were purchased from Thermo Fisher Scientific (VIC, Australia), and HPLC vials (1 mL) were bought from Agilent technologies (VIC, Australia).

Sample preparation

Fresh leaves of *Prosopis farcta* (Banks & Sol.) J.F.Macbr. plants were collected from Tehran, Iran, in July 2018. A botanist from the Alborz University of Medical Sciences of Iran identified taxonomically the plants. This specimen with the code IMPH-724 is kept in the Phytochemistry Research Center at Shahid Beheshti University of Medical Sciences, Tehran, Iran. The leaves were manually washed three times and dried at 25 ± 5 °C for one week. Samples were powdered by a mechanical grinder and were stored at a refrigerated temperature in a dark environment to avoid light exposure.

Extraction of phenolic compounds

Of the 400g of the powder, the sample was mixed with 800 mL of 85% ethanol and homogenized at 10,000 rpm for the 30s through the IKA Ultra-Turrax T25 homogenizer (Rawang, Selangor, Malaysia) and subjected to a shaking water bath (90 rpm) at 25°C for 24h. After that, the extracts were centrifuged with Hettich Refrigerated Centrifuge (ROTINA380R, Tuttlingen, Baden-Württemberg, Germany) at 5,000 rpm for 15 min. The Whatman No. 1 filter paper was used to filter the extract, followed by concentrated with a rotary evaporator (Laborota 4000, Heidolph, Germany) at 40 °C for 30 min. This process is repeated three times to achieve maximum extraction with ethanol. Finally, the extraction was stored at 4 °C for further analysis.

Estimation of phenolics and antioxidant potential

TPC, TFC, and TTC were measured for polyphenol estimation, while four different types of antioxidant assays, including TAC, DPPH, FRAP, and ABTS were performed for measuring their antioxidant capacity by adopting the previously published methods of Tang et al. (2019). A Multiskan® Go microplate photometer

(Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the data.

Determination of total phenolic content (TPC)

Folin-Ciocalteu's method is used to measure the total phenolic content of *P. farcta* samples. 25 µL of *P. farcta* extract, 200 µL of water, and 25 µL of Folin–Ciocalteu reagent solution (1:3 diluted with water) was added to a 96 well plate (Corning Inc., Midland, NC, USA) followed by incubation at room temperature for 5 minutes. Then, 25 µL of 10% (w/w) sodium carbonate was added and incubated again for 1 hour at room temperature in a dark environment. The absorbance was then measured at 760 nm through a spectrophotometer plate reader (Thermo Fisher Scientific, Waltham, MA, USA). The standard curve constructed by gallic acid was used to quantify the total phenolic content of samples with concentrations from 0 – 200 µg/mL. The results were described as mg of gallic acid equivalents (GAE) per g dry weight (dw) (mg GAE/g dw) of the sample.

Determination of total flavonoids content (TFC)

The aluminum chloride colorimetric method is used to estimate the total flavonoids of *P. farcta* samples. 80 µL of *P. farcta* extract was mixed with 80 µL of 2% (w/v) aluminum chloride solution and 120 µL of 50 g/L sodium acetate solution in a 96-well plate and incubated at room temperature for 2.5 h. The absorbance was determined at 415 nm. By using the standard curve made with quercetin (0 – 50 µg/mL), results were expressed as mass (mg) of quercetin equivalents (QE) per weight of the sample.

Determination of total tannins content (TTC)

25 µL of extract, 150 µL 4% (w/v) methanolic vanillin solution, and 25 µL 32% (v/v) sulfuric acid are mixed in a well plate, and then the mixture is incubated at 25°C for 15 min. The absorbance was conducted at a wavelength of 500nm. Through the standard curve generated from catechin at the range of 0 - 1000 µg/mL, the results were described as mass (mg) of catechin equivalents (CE) per weight of the sample.

Determination of total antioxidant capacity (TAC)

40 µL of the extract was mixed with 260 µL of phosphomolybdate regent (0.6 M H_2SO_4, 0.028 M sodium phosphate, and 0.004 M ammonium molybdate) (16). After being incubated at 95°C for 10 min, the mixture is cooled at 25°C. And then, the absorbance is determined at 695 nm. The standard curve is conducted by ascorbic acid at the range of 0 to 200 µg/mL, and the result is calculated by the standard curve and is described as mg/AAE per weight of the sample.

Determination of 2,2'-Diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay

25 µL of extract and 260 µL of 0.1 M DPPH radical methanol solution were added into a 96-well plate, followed by incubated at 25 °C for 30 min. After being incubated for half an hour in the dark at 25°C, the absorbance was determined at 517 nm. A standard curve was generated by the ascorbic acid aqueous solution at the range of 0 - 50 µg/mL. The results were expressed as mass (mg) of ascorbic acid equivalents (AAE) per weight of the sample.

Determination of ferric reducing antioxidant power (FRAP) assay

The FRAP reagent was produced by the mixture of 10 mM TPTZ solution, 300 mM acetate buffer, and 20 mM ferric chloride (the ratio is 1:1:10). After that, 280 µL FRAP reagent and 20 µL extracts were added in a 96 well plate and incubated at 37 °C for 10 min. The absorbance was determined at 593 nm. A standard curve was conducted using ascorbic acid at the range of 0 - 50 µg/mL. The results were expressed as mass (mg) of AAE per weight of the sample.

Determination of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

5 mL of 7 mM of ABTS solution were mixed with 88 µL of 140 mM potassium persulfate solution to produce the ABTS+ dye, followed by incubated in the dark at 25 °C for 16 h to prepare an ABTS+ free radical solution. Besides, ABTS+ stock solution was generated by diluted with ethanol to gain absorbance of 0.70 at 734 nm. After that, 10 µL extract and 290 µL of freshly prepared ABTS+ solution were mixed in a 96-well plate and incubated at room temperature for 6 min. The absorbance was determined at 734 nm. The standard curve was conducted by the concentrations of 0 - 150 µg/mL ascorbic acid, and the results were described as mass (mg) of AAE per weight of the sample.

Characterization of phenolic compounds using LC-ESI-QTOF-MS/MS analysis

The phenolic compound characterization was performed on an Agilent 1200 HPLC with an Agilent 6520 Accurate- Mass Q-TOF LC/MS (Agilent Technologies, Santa Clara, CA, USA) (16). The separation was carried out through a Synergi Hydro-RP 80 Å, reverse phase column (250 mm x 4.6 mm, 4 µm particle size) with protected C18 ODS (4.0 × 2.0 mm) guard column (Phenomenex, Lane Cove, NSW, Australia). Mobile phase A consisted of water/acetic acid (98:2, v/v), while mobile phase B was prepared in the ratio of acetonitrile/acetic acid/ water (50:0.5:49.5, v/v/v). The gradient profile was performed by a mixture of mobile phase A and B as follows: 10 –25% B (0 – 25 min), 25 – 35% B (25 – 35 min), 35 – 40% B (35 – 45 min), 40 – 55% B (45 – 75 min), 55 – 80% B (75 – 79 min), 80 – 90% B (79 – 82 min), 90 – 100% B (82 – 84 min), 100 – 10% B (84 – 87 min), isocratic 10% B (87 – 90 min). A 6 µL of the extract was injected, and the flow rate was set at 0.8 mL/min. Peaks identification was performed in both positive and negative ion modes with the capillary and nozzle voltage set to 3.5 kV and 500 V, respectively. Besides, the following conditions were maintained; *i)* nitrogen gas temperature at 300°C, *ii)* sheath gas flow rate of 11 L/min at 250°C, *ii)* nitrogen gas nebulization at 45 psi. A complete mass scan at the range of 50 to 1300 m/z was used, followed by carrying out the MS/MS analyses in automatic mode with collision energy (10, 15, and 30 eV) for fragmentation. The peak was identified in both positive and negative modes while the instrument control, data acquisition, and processing were performed through LC-ESI-QTOF-MS/MS MassHunter Workstation software (Qualitative Analysis, ver-

sion B.03.01, Agilent Technologies, Santa Clara, CA, USA).

Quantification of phenolic compounds using HPLC-PDA

An Agilent 1200 HPLC equipped with a photodiode array (PDA) detector was used to measure the quantification of targeted phenolic compounds by adopting the protocol (18). The condition and column are the same as that of LC-ESI-QTOF-MS/MS analysis. The twenty most abundant phenolic compounds present in the *P. farcta* samples, which includes 10 phenolic acids and 10 flavonoids, were selected for quantification purposes. The phenolic compounds were measured at three different wavelengths (280, 320, and 370 nm). The calibration standard curve was used to determine the quantification of targeted polyphenols, and the result was described as mg/g of sample. Agilent MassHunter Workstation software (Agilent Technologies, Santa Clara, CA, USA) was used to perform data collection.

Statistical analysis

The analyses are performed in triplicate, while the mean, standard deviation (n=3) is used to express the results. One-way analysis of variance (ANOVA) and Tukey's honestly significant differences (HSD) multiple rank test at $p \leq 0.05$ were used to verify the mean differences. ANOVA was conducted by Minitab for Windows version 19.0 (Minitab, LLC, State College, PA, USA) and GraphPad Prism 7.05 software for Windows (GraphPad 7.05 Software, San Diego, CA, USA, www.graphpad.com).

Results and Discussion

Polyphenol estimation (TPC, TFC, and TTC)

P. farcta leaves are rich in polyphenols, which include phenolic acids, flavonoids, and tannins. *P. farcta* samples phenolic contents were measured by TPC, TFC, and TTC, while the results were expressed as gallic acid equivalents, quercetin equivalent, and catechin equivalent, respectively.

Based on Table 1, the TPC value of *P. farcta* samples was 16.47 ± 1.63 mg GAE/g, which was lower than the study of García-Andrade et al. (2013) that they used aqueous acetone for extraction (5). Therefore, 85% of ethanol may be less sensitive than aqueous acetone in the extraction of *P. farcta* leaves. The total phenolic was estimated by Folin-Ciocalteu reagent, which could react with both phenolic and non-phenolic compounds, such as ascorbic acid and other reducing compounds (16). Those non-phenolic reducing compounds might also contribute to the high total polyphenol content of *P. farcta* samples. Therefore, advanced analytical techniques, including LC-ESI-QTOF-MS/MS, are significant in determining polyphenol characterization.

Flavonoids measured by the aluminum chloride colorimetric method in this research are an important group of phenolic compounds in the plants. The mechanism of method is that the carbonyl groups in flavonoids can be reacted with aluminum chloride to form a stable complex (17). The result of TFC in *P. farcta* samples was 0.21 ± 0.01 mg QE/g, which is similar to the result of Cardozo et al. (1). Besides, the efficiency of the extraction of flavonoids can be influenced by different extraction conditions, including temperature, extraction time, the type of solvents, solvent concentration, and so on (19).

Tennis, which can be divided into hydrolyzable tannins and condensed tannins, can be determined by methanolic vanillin solution. The TTC value of *P. farcta* was 2.63 ± 0.27 mg CE/g, following the previous studies (20).

Antioxidant potential (DPPH, ABTS, FRAP, and TAC)

To further study the antioxidant potential of the *P. farcta*, several antioxidant assays with different mechanisms were used. DPPH and ABTS were applied to estimate the radical scavenging ability, while FRAP and TAC assays were applied to measure reducing power of samples. The results were demonstrated in Figure 1.

The DPPH assay can be applied to measure the free radical scavenging activity (14). The mechanism of DPPH is that through the hydrogen-donating antioxidant, the stable free radical DPPH is reduced, and the non-radical form DPPH-H was formed. According to Figure 1, the DPPH radical scavenging ability of *P. farcta* leaves in this study was lower than in Lakshmibai et al. (21). The reason might be that the DPPH radical scavenging ability was based on both the presence of phenolic compounds and flavonoids (21). According to the TFC assay in our study, the value of the DPPH assay might be significantly influenced by the contents of flavonoids. Furthermore, the DPPH assay can also determine scavenged free radicals from non-phenolic compounds, such as ascorbic acid. Therefore, the an-

Table 1. Polyphenol estimation in *P. farcta*.

TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg CE/g)
16.47 ± 1.63	0.21 ± 0.01	2.63 ± 0.27

All data are presented as mg/g mean ± standard deviation (n = 3) and measured on a dry weight basis. TPC, Total phenolic content; TFC, total flavonoid content; TTC, total tannins content; GAE, gallic acid equivalents; CE, catechin equivalents; QE, quercetin equivalents.
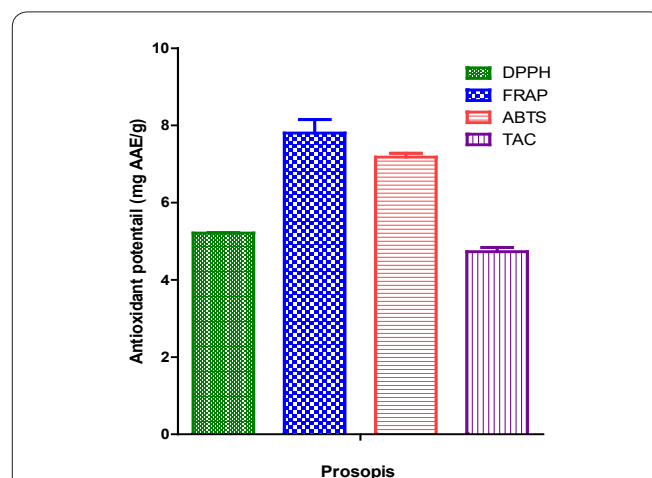

Figure 1. Antioxidant potential of *P. farcta* extract. DPPH, 2,2'-Diphenyl-2-picryl-hydrazyl; FRAP, Ferric Reducing-Antioxidant Power; ABTS, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); TAC, Total Antioxidant Capacity. Data of antioxidant activities in *P. farcta* are presented as mg ascorbic acid (AAE) equivalents per gram of samples (mg AAE/g).

Table 2. Characterization of phenolic compounds in *P. farcta* extract by LC-ESI-QTOF-MS/MS.

No.	Proposed compounds	Molecular Formula	RT (min)	Mode of ionization	Molecular Weight	Theoretical (*m/z*)	Observed (*m/z*)	Error (ppm)	MS/MS Product ion
Phenolic acid									
Hydroxybenzoic acids									
1	2-Hydroxybenzoic acid	$C_7H_6O_3$	11.294	** [M-H]⁻	138.0304	137.0231	137.0233	1.46	93
2	2,3-Dihydroxybenzoic acid	$C_7H_6O_4$	13.223	[M-H]⁻	154.0257	153.0184	153.0184	0.00	109
3	4-Hydroxybenzoic acid 4-*O*-glucoside	$C_{13}H_{16}O_8$	16.511	[M-H]⁻	300.0821	299.0748	299.0752	1.34	255, 137
Hydroxycinnamic acids									
4	Caffeic acid	$C_9H_8O_4$	15.871	[M-H]⁻	180.0417	179.0344	179.0341	-1.68	143, 133
5	Caffeic acid 3-*O*-glucuronide	$C_{15}H_{16}O_{10}$	19.297	[M-H]⁻	356.0724	355.0651	355.0653	0.56	179
6	Ferulic acid 4-*O*-glucuronide	$C_{16}H_{18}O_{10}$	19.475	[M-H]⁻	370.0882	369.0809	369.0808	-0.27	193
7	*m*-Coumaric acid	$C_9H_8O_3$	29.25	[M-H]⁻	164.0459	163.0386	163.0387	0.61	119
8	1,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	49.182	[M-H]⁻	516.1268	515.1195	515.1188	-1.40	353, 335, 191, 179
9	Sinapic acid	$C_{11}H_{12}O_5$	22.639	[M-H]⁻	224.068	223.0607	223.0605	-0.90	205, 163
Hydroxyphenylacetic acids									
10	3,4-Dihydroxyphenylacetic acid	$C_8H_8O_4$	12.671	[M-H]⁻	168.0412	167.0339	167.0337	-1.20	149, 123
Hydroxyphenylpropanoic acids									
11	Dihydroferulic acid 4-*O*-glucuronide	$C_{16}H_{20}O_{10}$	23.973	[M-H]⁻	372.1052	371.0979	371.0977	-0.54	195
12	Dihydrocaffeic acid 3-*O*-glucuronide	$C_{15}H_{18}O_{10}$	19.003	[M-H]⁻	358.0887	357.0814	357.0817	0.84	181
Hydroxyphenylpentanoic acids									
13	5-(3'-Methoxy-4'-hydroxyphenyl)-γ-valerolactone	$C_{12}H_{14}O_4$	24.916	[M+H]⁺	222.0874	223.0947	223.0946	-0.45	205
Flavonoids									
Flavanols									
14	4'-*O*-Methylepigallocatechin	$C_{16}H_{16}O_7$	10.052	[M+H]⁺	320.0882	321.0955	321.0963	2.49	302
15	Procyanidin dimer B1	$C_{30}H_{26}O_{12}$	16.511	** [M-H]⁻	578.1389	577.1316	577.1319	0.52	451
16	Myricetin 3-*O*-galactoside	$C_{21}H_{20}O_{13}$	16.784	** [M-H]⁻	480.0892	479.0819	479.0818	-0.21	317
17	Myricetin 3-*O*-rhamnoside	$C_{21}H_{20}O_{12}$	17.472	** M-H]⁻	464.0922	463.0849	463.0844	-1.08	317
18	Procyanidin trimer C1	$C_{45}H_{38}O_{18}$	19.062	[M-H]⁻	866.2041	865.1968	865.1972	0.46	739, 713, 695
19	(+)-Catechin	$C_{15}H_{14}O_6$	19.19	** [M-H]⁻	290.0771	289.0698	289.0701	1.04	245, 205, 179
20	(+)-Catechin 3-*O*-gallate	$C_{22}H_{18}O_{10}$	19.28	[M+H]⁺	442.0904	443.0977	443.0968	-2.03	289, 169, 125
Flavones									
21	Apigenin 6,8-di-C-glucoside	$C_{27}H_{30}O_{15}$	21.709	** [M-H]⁻	594.1592	593.1519	593.1516	-0.51	503, 473
22	6-Hydroxyluteolin 7-*O*-rhamnoside	$C_{21}H_{20}O_{11}$	25.27	** [M-H]⁻	448.1007	447.0934	447.0935	0.22	301
23	Rhoifolin	$C_{27}H_{30}O_{14}$	27.01	** [M-H]⁻	578.1645	577.1572	577.1573	0.17	413, 269
24	Apigenin 6-C-glucoside	$C_{21}H_{20}O_{10}$	28.242	** [M-H]⁻	432.1056	431.0983	431.0985	0.46	413,341,311
25	Diosmin	$C_{28}H_{32}O_{15}$	29.194	** [M-H]⁻	608.1723	607.1650	607.1653	0.49	301, 286
26	Chrysoeriol 7-*O*-glucoside	$C_{22}H_{22}O_{11}$	34.825	[M+H]⁺	462.1158	463.1231	463.1227	-0.86	445,427,409,381

Flavanones

27	Neoeriocitrin	$C_{27}H_{32}O_{15}$	21.37	[M-H]⁻	596.1726	595.1653	595.1649	-0.67	431, 287
28	Hesperetin 3'-*O*-glucuronide	$C_{22}H_{22}O_{12}$	44.721	[M-H]⁻	478.1111	477.1038	477.1031	-1.50	301, 175, 113,85

Flavonols

29	Quercetin 3-*O*-(6"-malonyl-glucoside)	$C_{24}H_{22}O_{15}$	22.405	[M+H]⁺	550.0911	551.0984	551.0983	-0.18	303
30	Myricetin 3-*O*-rutinoside	$C_{27}H_{30}O_{17}$	25.561	[M-H]⁻	626.1487	625.1414	625.1421	1.12	301
31	Kaempferol 3-*O*-(2"-rhamnosyl-galactoside) 7-O-rhamnoside	$C_{33}H_{40}O_{19}$	25.686	** [M-H]⁻	740.2189	739.2116	739.2118	0.27	593, 447, 285
32	Kaempferol 3,7-*O*-diglucoside	$C_{27}H_{30}O_{16}$	28.515	[M-H]⁻	610.1521	609.1448	609.1451	0.49	447, 285

Dihydrochalcones

33	3-Hydroxyphloretin 2'-*O*-glucoside	$C_{21}H_{24}O_{11}$	17.087	[M-H]⁻	452.1297	451.1224	451.1226	0.44	289, 273
34	Phloridzin	$C_{21}H_{24}O_{10}$	31.13	[M-H]⁻	436.1332	435.1259	435.1263	0.92	273

Dihydroflavonols

35	Dihydromyricetin 3-*O*-rhamnoside	$C_{21}H_{22}O_{12}$	19.604	[M-H]⁻	466.1092	465.1019	465.1021	0.43	301
36	Dihydroquercetin 3-*O*-rhamnoside	$C_{21}H_{22}O_{11}$	22.819	** [M-H]⁻	450.1162	449.1089	449.109	0.22	303

Isoflavonoids

37	6"-*O*-Acetylglycitin	$C_{24}H_{24}O_{11}$	45.345	[M+H]⁺	488.1319	489.1392	489.1378	-2.90	285,270
38	5,6,7,3',4'-Pentahydroxyisoflavone	$C_{15}H_{10}O_{7}$	29.837	[M+H]⁺	302.0417	303.0490	303.049	0.00	285, 257
39	6"-*O*-Malonylgenistin	$C_{24}H_{22}O_{13}$	31.447	** [M+H]⁺	518.1039	519.1112	519.1114	0.39	271
40	3'-Hydroxygenistein	$C_{15}H_{10}O_{6}$	32.84	[M+H]⁺	286.047	287.0543	287.0544	0.35	269, 259
41	Glycitin	$C_{22}H_{22}O_{10}$	35.318	[M+H]⁺	446.1196	447.1269	447.1274	1.12	285

Other polyphenols
Hydroxycoumarins

42	Coumarin	$C_{9}H_{6}O_{2}$	9.554	[M+H]⁺	146.0374	147.0447	147.0445	-1.36	103, 91

Hydroxybenzaldehydes

43	4-Hydroxybenzaldehyde	$C_{7}H_{6}O_{2}$	19.809	** [M-H]⁻	122.036	121.0287	121.0287	0.00	77

Hydroxyphenylpropenes

44	2-Methoxy-5-prop-1-enylphenol	$C_{10}H_{12}O_{2}$	30.612	[M+H]⁺	164.0827	165.0900	165.0896	-2.42	149, 137, 133, 124

Tyrosols

45	Hydroxytyrosol 4-*O*-glucoside	$C_{14}H_{20}O_{8}$	24.382	[M-H]⁻	316.1148	315.1075	315.1078	0.95	153, 123

Lignans Schisandrol B

46		$C_{23}H_{28}O_{7}$	5.947	[M+H]⁺	416.184	417.1913	417.1914	0.24	224, 193, 165

Stilbenes

47	3'-Hydroxy-3,4,5,4'-tetramethoxystilbene	$C_{17}H_{18}O_{5}$	30.612	[M+H]⁺	302.1155	303.1228	303.1217	-3.63	229, 201, 187, 175

tioxidant potentials of polyphenols cannot be accurately measured when only using the DPPH assay.

Through the hydrogen atom donating tendency of phenolic compounds, the ABTS assay is another method that can be applied to estimate the antiradical scavenging abilities. A colorimetric assay, where antioxidants in samples reduce ABTS+ and form a stable free radical, was used to determine the scavenged ABTS free radicals. The ABTS results in our research were in accordance with the previous studies (1). However, in the previous study, ABTS results were similar to that of the DPPH assay (16). In this study, the difference between the results of ABTS and DPPH might be caused by the complex structure of phenolic compounds in *P. farcta*, which resulted in an underestimation of DPPH scavenging activities (17).

The mechanism of the FRAP assay is the reduction of Fe^{3+} to Fe^{2+}. During the reduction reaction, both electron transfer from the sample or antioxidant and the ability of the extracts to act as antioxidants via donating electrons could be developed with the rise of absorbance. The high value of FRAP assay in this study demonstrated the high ferric reducing power, which may be related to the TPC value.

Based on the electron transfer, the mechanism of total antioxidant capacity (TAC) assay is that antioxidant or phenolic compounds can reduce molybdenum (VI) to molybdenum (V), which is similar to FRAP. However, the TAC assay result in our study was significantly lower than that of the FRAP assay (p<0.05). Therefore, LC-ESI-QTOF-MS/MS was important to be used for further research.

LC-ESI-QTOF-MS/MS characterization of the phenolic compounds

LC-MS/MS has been popularly known to identify and characterize phenolic compounds present in the plant species (22). The Qualitative analysis of *P. farcta* phenolic compounds of leaves has been achieved by using LC-ESI-QTOF-MS/MS. phenolic compounds of *P. farcta* leaves were tentatively characterized from their *m/z* value, and MS spectra in both modes of ionization, including positive and negative. The software used was Agilent LC-MS qualitative software and personal compound database and library (PCDL). Compounds with mass error < ±10ppm and PCDL library score > 80 were selected to further analyze MS/MS identification and *m/z* characterization. The results were shown in Table 2 and the structures of compounds in the figure 2.

In this study, LC-MS/MS has characterized 47 compounds, including phenolic acids (13), flavonoids (28), other polyphenols (4), lignans (1), and stilbenes (1).

Phenolic acids

Phenolic acids are widely distributed plant phenolic compounds (23, 24). In our study, 13 phenolic acids were characterized, including hydroxybenzoic acids (3), hydroxycinnamic acids (6), hydroxyphenylacetic acids (1), hydroxyphenylpropanoic acids (2), and hydroxyphenylpentanoic acids (1).

Compound 3 was tentatively characterized in a negative mode of ionization with precursor ion at *m/z* 299.0748. On MS/MS analysis, 4-hydroxybenzoic acid 4-*O*-glucoside was characterized based on product ions at *m/z* 137, due to the loss of hexosyl moiety (162 Da) and *m/z* 255, due to loss of CO_2 (44Da) (25). In the previous study of Bento-Silva, Koistinen (26), the spices and herbs had a higher concentration of *4*-hydroxybenzoic acid-*O*-glucoside, including anise, caraway, coriander, fennel, and star anise. Fruits and vegetables have a low concentration of the compound (27), including blackberries, red raspberries and strawberries (26).

Compound 4 was identified as caffeic acid based on the precursor ion [M-H]⁻ at *m/z* 179.0341. Upon MS/MS, the compound was confirmed by the peak fragment at *m/z* 143 and *m/z* 133 due to the corresponding loss of two H_2O units (36 Da) and HCOOH (46 Da) from the precursor ion (28). Caffeic acid constituents one of the main hydroxycinnamic acid (29), present in both free and esterified form and is about 75 - 100% of the total content of hydroxycinnamic acid in fruits (30). Caffeic acid has attracted various researches due to its anticarcinogenic properties as it prevents the formation of nitro compounds, including nitrosamines and nitrosamides (31). Hepatocellular Carcinoma (HCC) is a malignant cancer that occurs in the liver and has the highest mortality as well as a short survival time (32). Caffeic acid can act in HCC by its antioxidant content by preventing ROS production and reducing oxidative stress. Caffeic acid act as a primary and secondary antioxidant (31). Caffeic acid was previously found in two species of *Prosopis,* including *P. cineraria* and *P. glandulosa* (33). Another study found caffeic acid in butanol fraction of hydroethanolic extract of P. cineraria (34). Elansary, Szopa (35) found to caffeic acid in tuber extract of *Asparagus aethiopicus* of tuber extract and leaf extracts of *Kalanchoe delagoensis*.

m-Coumaric acid (compound 7 with [M − H]⁻ *m/z* at 163.0387) with peak fragmentation at *m/z* 119 due to the consecutive loss of CO_2 (44 Da) (36). Coumaric acid had previously significant shown anti-inflammatory and antioxidant activities (37). *m*-coumaric acid was seen in the ethyl acetate extract of *Moringa oleifera* (38), *Heracleum persicum* (39), *and* P. spinosa fruit extract (40). m-Coumaric acid is also found in Dendropanax morbifera leaf extract (41), a traditional medicine used in treating headaches, dysmenorrhea, and skin diseases.

Compound 9 present in the negative ionization mode was tentatively identified as sinapic acid with precursor ion at *m/z* 223.0605. Upon MS/MS analysis, compound 9 was confirmed by the presence of product ions at *m/z* 205 (M-H-18, loss of H_2O) and *m/z* 163 (M-H-60, loss of two CH_2O) (42). Sinapic acid is one of the hydroxycinnamic acids and is present in fruits, vegetables, and herbs. The pharmacological properties include anti-inflammatory, antioxidant, anti-hyperglycemic, and hepatoprotective activities. Based on research, sinapic acid plays a role in increasing hair growth (43). The reactive oxygen species (ROS) generated by the UVB can alter the DNA, lipids, and proteins. But sinapic acid can protect the cells from UVB by reducing the cell toxicity and ROS generations (44). Osteoarthritis is a degenerative joint disease, IL-1β inflammatory cytokines are one reason for osteoarthritis, and sinapic acid can inhibit the production of nitric oxide and prostaglandin E2 (45). Previously, sinapic acid was found in lemon, orange, mango, avocado, strawberries, raspberries, apple, pears, cabbage, radish, and broccoli (46). It was also reported

in spices and herbs, including anise, nutmeg, thyme, and basil (47).

Flavonoids

Flavonoids are a subclass of phenolic compounds with antioxidant and free radical scavenging properties (48). On LCMS/MS analysis, 28 flavonoids were characterized, including flavanols (7), flavones (6), flavanones (2), flavonols (4), dihydrochalcones (2), dihydroflavonols (2), and isoflavonoids (5).

(+)-Catechin was identified in both modes of ionization at m/z 289.0701. Further analysis of MS/MS confirmed the presence of the compound 19 based on the product ions at m/z 245 (M-H-44, loss of CO_2), m/z 205 (M-H-84, loss of flavonoid A ring), m/z 179 (M-H-110, loss of flavonoid B ring) (25). Catechins are procured from various sources, including fresh tea leaves, apricots, strawberries, wines, cider, red wine, green and red algae (49). Many medicinal plants that have catechin including *Ficus mucuso* from Africa, Thalassodendron ciliatum from Egypt, Peltophorum africanum from South Africa (50). Catechins can regulate gene and protein expression in neurons and be therapeutic drugs for aging and related diseases (51).

Compound 23 present in both modes identified as rhoifolin with precursor ion at ([M-H]$^-$ at m/z 577.1573). MS/MS analysis confirmed compound rhoifolin by the presence of the product ions at m/z 413 and m/z 269 due to the corresponding loss of rhamnose moiety and H_2O (164 Da) and hexosyl moiety plus rhamnose moiety (308 Da) (52). Rhoifolin belonged to apigenin's family and was the first time found in *Rhus succedanea* in 1952 (53). The biological activities include anti-inflammatory, anticancer, antidiabetic, antimicrobial (54). Rhoifolin isolated from the plants has the potential to be the drug candidate against COVID-19 (55). Brinza, Abd-Alkhalek (56) studied that rhoifolin can be a compound used against anxiety and amnesia by restoring cholinergic activity and the amelioration of brain oxidative stress. Rhoifolin present in the plant samples, including bitter orange, lemon, bananas, tomatoes, and grapes (54). Rhoifolin isolated from the leaves of *Chorisia crispiflora* had biological activity against the carcinoma cell lines of the hepatocellular, colon and, human fetal lung fibroblast. This compound is highly recommended as it has no negative effect against normal healthy cells (57).

Other polyphenols

In our study, 4 other polyphenols were characterized, including hydroxycoumarins (1), hydroxybenzaldehyde (1), hydroxyphenylpropenes (1), and tyrosols (1).

Coumarin (Compound 42) was identified in a positive ionization mode and was tentatively identified with precursor ion [M-H]$^+$ at m/z 147.0445. The peak fragmentation at m/z 103 [M + H −44, loss of CO2] and m/z 91 [M + H −56, loss of 2CO] (58). Coumarin is a natural product found in plants and is known to have a vanilla-like odor (59). In the early 1960s, coumarin was isolated from a South American plant seed known as *Dripteryx odorata* (60). Coumarin has various biological activities, including anti-inflammatory, antimicrobial, anticoagulant, and anti-helminthic (61). Previously found in African medicinal plants, including

twigs of *Dorstenia elliptica*, the bark of *C. grevei* Baill, roots of *T. asiatica,* and leaves of *C. anisate* (59). The compound coumarin extracted from Calophyllum brasiliense and Mammea americana is Mexican medicinal plants that showed higher activity against Staphylococcus aureus (62). Coumarin and derivatives have been developed as a modulator for the anti-tuberculosis drugs, which is clinically approved therapeutic targets as it shows therapeutic potency (63).
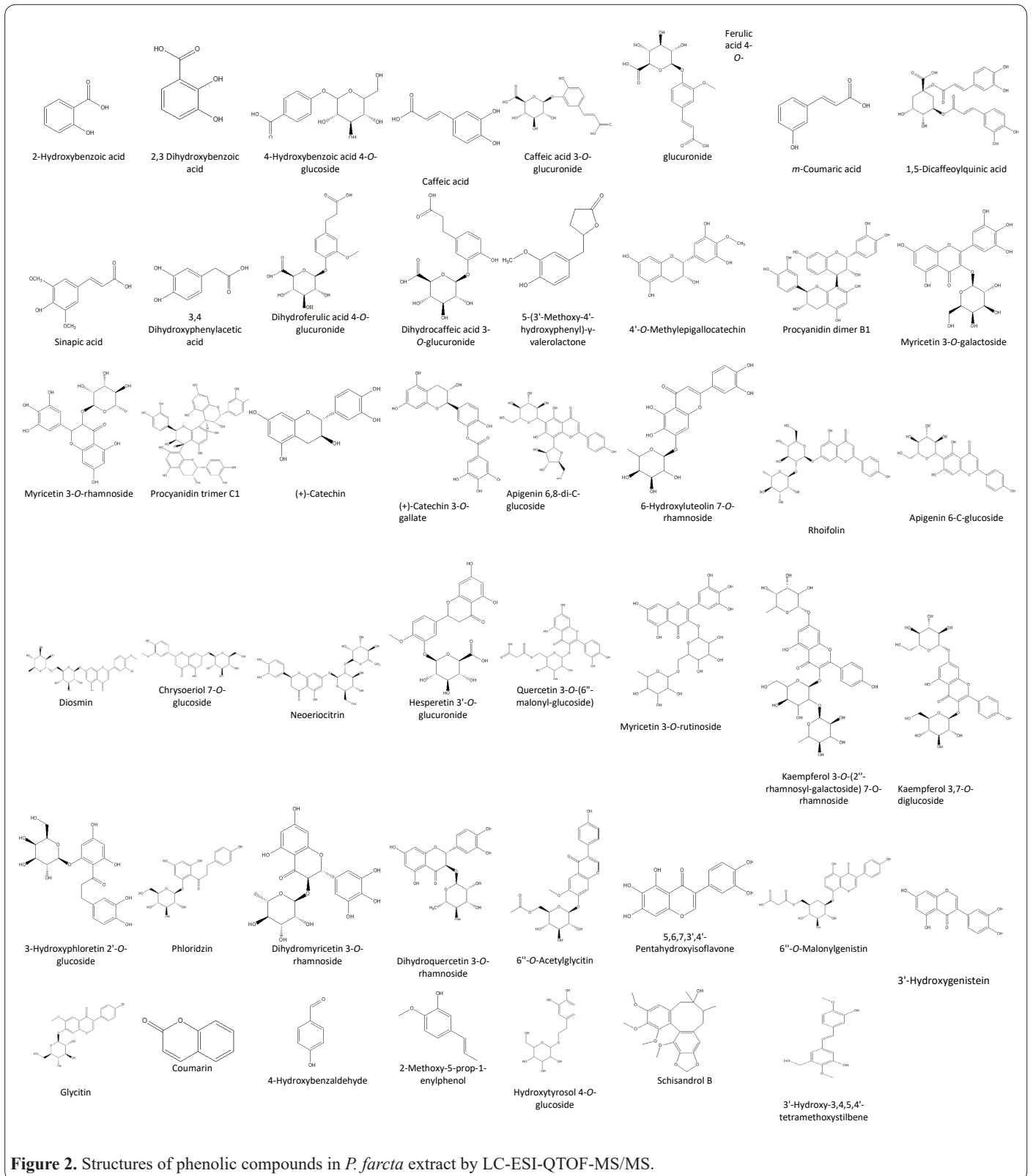
HPLC Quantification

HPLC is an effective technique to determine polyphenol content and chemical compounds of different varieties of plants (64). In this research, 10 targeted polyphenols, including phenolic acids and flavonoids, were determined by their UV spectra (Figure 3). After that, the retention times of those polyphenols were compared with reference standards. Based on the results of both LC-ESI-QTOF-MS/MS and HPLC-PDA, the main phenolic compounds in *P. farcta* samples were flavonoids, which had a higher compound diversity.

The main phenolic acids in *P. farcta* samples were chlorogenic acid (9.78 ± 2.15 mg/g dw), which had a significantly higher concentration than any other phenolic acids. Moreover, caffeic acid (3.17 ± 1.30 mg/g dw), p-Hydroxybenzoic acid (3.17 ± 1.10 mg/g dw), protocatechuic acid (2.18 ± 1.09 mg/g dw), and gallic acid (1.39 ± 0.27 mg/g dw) were also important phenolic acids in *P. farcta*. In the previous study, protocatechuic acid, chlorogenic acid, caffeic acid, and ferulic acid in *P. cineraria* were quantified by HPLC-DAD, which was a similar technique to quantitative detect the phenolic compounds in butanol fractions of PC hydroethanolic extract (BFPC) (34). Besides, the significantly higher values of the results from the research of Yadav et al. (2018) also demonstrated that butanol fractions of PC hydroethanolic extract might be more sensitive than 85% ethanol in the extraction of *P. farcta* leaves

According to Figure 2, *P. farcta* also had high contents of flavonoids. Catechin (12.73 ± 1.29 mg/g dw) was the most abundant flavonoid in *P. farcta*, which was followed by kaempferol and quercetin, with (7.93 ± 1.47 mg/g dw) and (5.78 ± 1.13 mg/g dw), respectively. Epicatechin (2.86 ± 1.24 mg/g dw) and epicatechin gallate (1.89 ± 1.74 mg/g dw) had relatively low concentrations. According to the previous research, apigenin 6-C-glucoside, quercetin-3-galactoside, and luteolin in *P. farcta* were determined by HPLC, and the phenolic composition was affected by the geographical origin, the nature of the cultivar, and cold acclimation (65). Moreover, anthocyanidins, such as cyanidin 3-hexoside, cyanidin malonyl hexoside, and peonidin malonyl hexoside, were detected in *P. chilensis* samples via HPLC (66). However, those anthocyanidins cannot be detected in *P. farcta* samples in our study, which meant that Prosopis's different genera might contain other flavonoids.

LC-ESI-QTOF-MS/MS analysis was used to identify and characterize phenolic compounds from *P. farcta* leaves. As a result, 47 phenolic compounds were determined, according to their m/z value and mass spectrometric spectra under both positive and negative ionization modes. The polyphenol classes could be divided into phenolic acid, flavonoids, other polyphenols, lignans, and stilbenes, while rhoifolin was a significant type of

Figure 2. Structures of phenolic compounds in *P. farcta* extract by LC-ESI-QTOF-MS/MS.
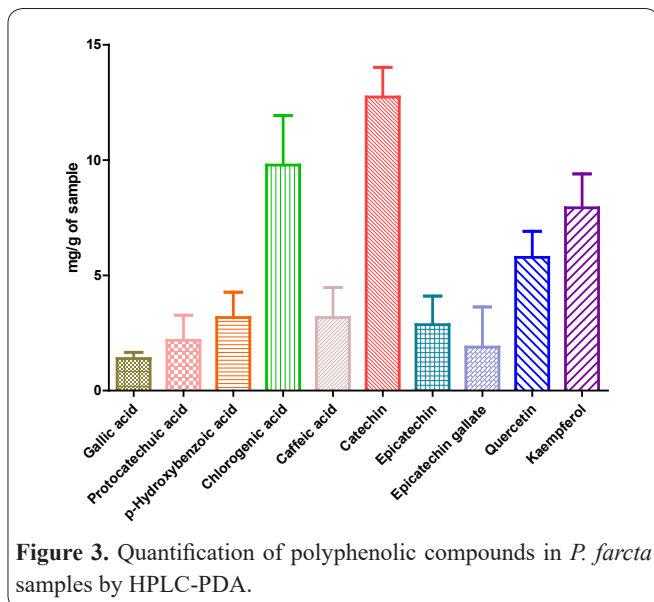
Figure 3. Quantification of polyphenolic compounds in *P. farcta* samples by HPLC-PDA.

flavonoid that deserved attention due to its potential against COVID-19. Besides, HPLC-PDA analysis was applied to quantify 10 individual polyphenols by comparing their UV spectra and the retention times with reference standards. Consequently, *P. farcta* samples were rich in phenolic acids and flavonoids, while catechin was the most abundant. Four types of antioxidant assays were used to estimate *P. farcta* samples' antioxidant capacity, which demonstrated that antioxidant capacity is related to phenolic content. Overall, *P. farcta* leaves can be a promising source of natural phenolic compounds. And their strong antioxidant capacity supports their potential applications in food, beverages, feeds, nutraceuticals and pharmaceutical products.

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