Heliyon 10 (2024) e25496

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Analyses of phytochemical compounds in the flowers and leaves of *Spiraea japonica* var. *fortunei* using UV-VIS, FTIR, and LC-MS techniques

Semra Yılmazer Keskin [a,*], Ayşe Avcı [b], Haka Fajriana Febda Kurnia [a,c]

[a] Department of Chemistry, Sakarya University, Sakarya, Turkiye
[b] Department of Food Engineering, Sakarya University, Sakarya, Turkiye
[c] Institute of Natural Sciences, Sakarya University, Sakarya, Turkiye

ARTICLE INFO

Keywords:
Spiraea japonica var. *fortunei*
Phytochemicals
Phenolic compounds
Flavonoids
FTIR
LC-MS

ABSTRACT

Spiraea japonica var. *fortunei* has been extensively used in traditional Chinese medicine and is well-known for its alkaloids. However, there is no adequate study concerning the phenolic compounds. Therefore, this study aimed to investigate phenolic compounds found in the leaves and the flowers of the plant both qualitatively and quantitatively. Extractions were performed either with ethanol or methanol, and methanol has shown better performance than ethanol. The leaves were a better source of phenolic compounds than the flowers. The total phenolic content of the methanol extract of the leaves was 25.64 ± 0.32 mg GAE/g dry matter, and antioxidant activity, as determined with the DPPH method, was 69.76 ± 0.34 %. UV-VIS spectrum and FTIR analyses confirmed the presence of phenolic compounds. The phenolic profile was investigated with LC-MS using both negative and positive ionization, and a total of 55 phenolic compounds that are attractive for pharmaceutical and medical applications were observed.

1. Introduction

Phenolic compounds are phytochemicals containing at least one aromatic ring with an attached hydroxyl group [1]. They are the secondary metabolites produced by many plants as defense mechanisms against insects, microorganisms, and dangerous chemicals [2]. Due to high biological activity, phenolic compounds are important in traditional and complementary medicine [3]. One of the most essential properties of phenolic compounds is that they possess an antioxidant property that helps prevent tissue injury by removing free radicals. Antioxidants can function as reducing agents, free radical scavenging, and singlet oxygen quencher [4]. There is an increasing interest in using natural antioxidants in foods as they are safer than synthetic ones [5]. Flavonoids are members of phenolics composed of a 15-carbon atom phenylpropanoid core structured into three rings: A, B (six carbon atoms aromatic rings), and C (heterocyclic ring containing three carbon atoms). They are together represented as C6–C3–C6 [6]. They include flavonols, flavanones, flavones, flavan-3-ols, and isoflavones. Flavonoids exhibit antioxidant and pro-oxidant properties due to the *ortho* or *para* hydroxyl group in the 2-phenyl ring and free hydroxyl at the 5,7- positions, respectively [2,4].

Genus *Spiraea* belongs to the Rosaceae family, which comprises more than 100 species. Species of *Spiraea* grow in moderate climate regions, especially in Asia [7,8]. *Spiraea japonica* is a perennial shrub with pink flowers widespread across East Asia. It is a complex of

* Corresponding author.
E-mail address: syilmazer@sakarya.edu.tr (S. Yılmazer Keskin).

https://doi.org/10.1016/j.heliyon.2024.e25496
Received 20 August 2023; Received in revised form 8 January 2024; Accepted 29 January 2024
Available online 1 February 2024

14 morphologically distinguished species depending on the leaf size and shape [9,10]. The plant is known to contain active compounds such as alkaloids and phenolics. It has been used as a diuretic, detoxifier, anti-inflammation, pain reliever, and for treating coughs, headaches, and toothaches in traditional Chinese medicine for a long time [11,12]. Alkaloids from *S. japonica* have been extensively studied during the last two decades [13]. However, to our knowledge, there was no study about the phenolic and antioxidant properties of the plant. Therefore, this study aimed to determine phenolic compounds in *S. japonica* var. *fortunei.* The leaves and the flowers of the plant were analyzed separately using two different extraction solvents (ethanol and methanol). Phytochemical constituents were determined quantitatively and qualitatively with UV-VIS spectrophotometer and Fourier transform infrared spectrometer. Phytochemical profiles were also examined with an LC-MS spectrometer.

2. Materials and methods

2.1. Materials

Spiraea japonica var. *fortunei* was collected (500 g) from Sakarya University Campus (40° 44′N, 30° 19′E, 217 m.a.s.l.) in August 2021. The flowers and leaves of the plant were dried separately in shadow at room temperature. Then, dried flowers and leaves were ground in a Waring blender and sieved (18 mesh) to have a fine powder. The samples were stored in plastic bags at room temperature until the analyses.

Sodium carbonate, methanol, ethanol, gallic acid, aluminum chloride, sodium nitrite, sodium hydroxide, and Folin Ciocalteau reagent were purchased from Merck (Darmstadt, Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (St. Louis, USA).

2.2. Preparation of extracts

Both methanol and ethanol extracts of leaves and flowers were prepared. For this, 0.25 g of powdered samples were weighed, and 10 mL of 80 % (*v/v*) alcohol was added. The samples were stirred moderately on a magnetic stirrer (120 rpm) at room temperature for 24 h for the extraction. Then, the solution was centrifuged at 9320×*g* for 10 min, the supernatant was collected for further analysis, and the marc was discarded. The extracts were stored at −30 °C until the analyses.

The clear filtrate was dried under a vacuum at 40 °C to calculate the extraction yield using a rotary evaporator (IKA KS 400, Switzerland). The resulting dry crude extract was weighed to calculate the extraction yield using the following equation (Eq. 1).

$$\text{Extraction yield } (\%) = \frac{W_1}{W_2} * 100 \quad \text{(Eq. 1)}$$

Where W_1 was the weight of the extract after evaporation, and W_2 was the amount of dried sample.

2.3. Determination of total phenolic contents

The total phenolic contents of the *S. japonica* var. *fortunei* extracts were determined by using the Folin Ciocalteau method [14]. For this, 25 μL of the extract was mixed with 75 μL of an appropriate solvent (80 % methanol or ethanol), 200 μL of Folin Ciocalteau reagent, and 2 mL of distilled water. Three minutes later, 1 mL of Na_2CO_3 solution (20 %, *w/v*) was added and allowed to stand for 1 h in the dark. At the end of the reaction, the absorbance of the sample was measured at 765 nm using a UV-VIS spectrophotometer (Agilent Model 8453 Diode Array Spectrophotometer). A standard curve was constructed using gallic acid (0–500 ppm). The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry matter of the sample [15].

2.4. Determination of total flavonoid content

Aluminium chloride colorimetric assay was used to determine the total flavonoid content [4]. Briefly, 100 μL of the extract was mixed with 300 μL of distilled water and 30 μL of $NaNO_2$ (5 %, *w/v*) and left to stand for 5 min at room temperature. Then, 30 μL of $AlCl_3$ (10 %, *w/v*) was transferred into the reaction mixture. After 5 min, 200 μL of NaOH (1 mM) was added. Finally, the volume was completed to 1 mL with distilled water and vortexed. The intensity of the orange-yellow color created by the flavonoids was measured at 510 nm using a UV-VIS spectrophotometer. Quercetin standard (10–800 ppm) was prepared in the same conditions to calculate the amount of flavonoids in the sample. The results were expressed as mg quercetin (QE)/g dry matter of the sample [16].

2.5. Determination of antioxidant activity by the DPPH method

Antioxidant activity was determined using DPPH radical scavenging activity with some modification [17]. Various amounts of extracts (2.5, 5, 10, 50, and 100 μL) were added to the test tube, and the volumes were completed to 200 μL with 70 % methanol (*v/v*). The samples were reacted with 3 mL of DPPH (0.051 mmol; in 70 % methanol) solution for 30 min at room temperature. Then, the decrease in the absorbance compared to the control was monitored at 517 nm. The control sample contained 200 μL of 70 % methanol. DPPH radical scavenging activity was calculated using the following equation (Eq. 2).

Table 1
Extraction yields of different parts of *Spiraea japonica* var. *fortunei* extracted with ethanol and methanol.

Plant material	Extraction Yield (%)*	
	Ethanol extract	Methanol extract
***S. japonica* var. *fortunei* flowers**	13.0 ± 1.2^{a}	17.1 ± 0.3^{c}
***S. japonica* var. *fortunei* leaves**	25.9 ± 0.9^{b}	32.5 ± 2.1^{d}

*The values represent the average ± standard deviation of three independents results. The significant differences between the samples were indicated with different letters ($P < 0.05$).

$$\text{DPPH radical scavenging activity } (\%) = \frac{(A_C - A_S)}{A_C} * 100 \quad \text{(Eq. 2)}$$

Where A_C was the absorbance of the control, A_S was the absorbance of the sample.

The regression equation of the relationship between sample concentration and % free radical inhibition is used to find the 50 % inhibitory concentration (IC_{50}) of free radical activity [18].

2.6. Identification experiments

2.6.1. UV-VIS analysis

The leaf and flower extracts that were obtained with different solvents were subjected to UV-VIS scanning analysis. They were diluted 30 times using the corresponding extraction solvent (ethanol or methanol) at ambient temperature. The absorbance spectra were recorded between 200 and 800 nm wavelengths with a UV-VIS spectrophotometer.

2.6.2. FTIR analysis

To determine functional groups associated with the phenolic compounds have been detected with the help of a Fourier Transform Infrared (FTIR) spectrometer with ATR technique (Shimadzu IR, Prestige 21, Nakagyo-ku, Japan). The extracts were dried using a rotary evaporator under a vacuum at 40 °C for the measurements. The spectra were obtained at room temperature in the range of 4000 and 400 cm^{-1}.

2.6.3. LC-MS analysis

The methanol extracts of the leaves and the flowers of *S. japonica* var. *fortunei* were analyzed using a Shimadzu LCMS-9030 spectrometer with a Q-TOF (Quadrupole-Time of Flight) analyzer. Electrospray Ionization (ESI) was applied at both positive and negative ionization modes, and the interface voltage was 3.0 kV. The mobile phase consisted of acetonitrile and formic acid at gradient mode. A CN column (3 μm, 15 cm, 4 mm) was used as the stationary phase. Acetonitrile and 30 mM formic acid solution were used as mobile phase in gradient mode. The linear gradient profile was: 0–8 min 5 % acetonitrile, 8–13 min 15 % acetonitrile, 13–18 min 30 % acetonitrile, 18–20 min 35 % acetonitrile, 20–24 min 60 % acetonitrile, 24–27 min 80 % acetonitrile, 27–30 min 90 % acetonitrile, 30–32 95 % acetonitrile, and 32–35 min 5 % acetonitrile [19]. The injection volume was 10 μL. The peaks obtained at both negative and positive ionization modes were analyzed using LCMS LabSolutions 5.109 software, and phenolic compounds were estimated. The analyses were performed at Sakarya University Research Development and Application Center.

2.7. Statistical analysis

All the analyses were performed in triplicate, and the results were expressed as average ± standard deviation. The data were statistically analyzed with analysis of variance (ANOVA) using SPSS (version 11.5, SPSS Inc., USA). Duncan's multiple range test with a significance level of 0.05 ($P < 0.05$) was applied to determine the differences between the samples.

3. Result and discussion

3.1. Effect of different solvents on the extraction yield

Extraction yield is a measure of the effectiveness of the solvent and the extraction method to extract certain components from the plants. The dried flowers and the leaves of the *S. japonica* var. *fortunei* having dry matters of 76.44 and 73.37 %, respectively, were extracted separately by using both ethanol and methanol to determine the effect of different solvents (Table 1). Extraction yields of *S. japonica* var. *fortunei* leaves were significantly higher than the flowers with both solvents ($P < 0.05$). The highest extraction yield was obtained from the leaves (32.5 ± 2.1 %) extracted with methanol, and the lowest from the flowers (13.0 ± 1.2 %) extracted with ethanol ($P < 0.05$). When methanol was used, the extraction yields of the flowers and the leaves were increased by 31.5 and 25.5 %. The extraction yield and quality of the extract are greatly influenced by the type of solvent, the characteristics of raw material, and the method used [20,21]. Various researchers reported different extraction yields from different raw materials. Mostafa et al. [22] used ethanol as the extraction solvent for five different plant species (*Syzygium aromaticum, Thymus vulgaris, Punica granatum, Zingiber officinales,* and *Cuminum cyminum*) and determined that the yields ranged from 3.12 to 9.74 %. Ethanol extract of *Hibiscus sabdariffa*

Table 2
Total phenolic and flavonoid contents of the extracts obtained with different solvents.

Plant Material	Solvent	Total Phenolic (mg GAE/g dry matter)*	Total Flavonoid (mg QE/g dry matter)*
***S. japonica* var. *fortunei* flowers**	Ethanol	11.08 ± 0.13^{b}	$16{,}98 \pm 3.07^{B}$
	Methanol	19.33 ± 1.24^{a}	22.68 ± 2.63^{A}
***S. japonica* var. *fortunei* leaves**	Ethanol	18.13 ± 1.61^{a}	46.46 ± 2.96^{C}
	Methanol	25.64 ± 0.32^{c}	28.35 ± 3.54^{A}

*The values represent the average ± standard deviation of three independents results. The significant differences between the samples were indicated with different letters ($P < 0.05$): lower case letters are for the total phenolic contents, and uppercase letters are for the flavonoid contents of the samples.

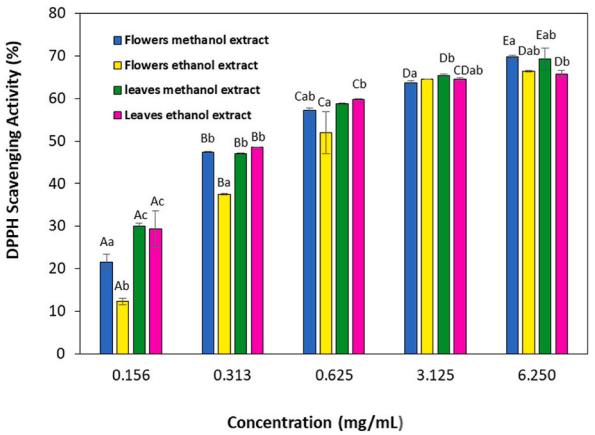

Fig. 1. Antioxidant activities of the extracts at varying concentrations determined by DPPH radical scavenging method. All the data were expressed as average ± standard deviation of three independent samples. Different letters indicate the significant statistical differences at $P < 0.05$. Lower case letters represent the significant differences between ethanol and methanol extracts of flowers and leaves at the same concentration; upper case letter represents the significant differences between the different sample concentrations.

was reported to be 21 % [23].

3.2. Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of the extracts are depicted in Table 2. Significant differences between the flavonoid contents of ethanol extracts of flowers and leaves have been detected ($P < 0.05$). On the other hand, there was no significant difference between the flavonoid contents of methanol extracts ($P > 0.05$). The lowest flavonoid content was observed with the ethanol extract of the flowers (16.98 ± 3.07 mg QE/g dry matter), while the highest (46.46 ± 2.96 mg QE/g dry matter) was with ethanol extract of the leaves. The total phenolic contents of the ethanol and methanol extracts of the flowers and leaves were significantly different ($P < 0.05$). The lowest total phenolic content was obtained with ethanol extracts of the flower (11.08 ± 0.13 mg GAE/g dry matter), and the highest was with methanol extracts of the leaves (25.64 ± 0.32 mg GAE/g dry matter). Similar total phenolic contents were determined with the methanol extracts of the flowers and the ethanol extracts of the leaves ($P > 0.05$). Shirshova et al. [24] investigated the total phenolic content of the leaves and flowers of *S. media* using the aqueous and ethanol extracts, and they obtained higher phenolic compounds in the leaves than in the flowers. Keskin et al. [3] have reported higher total phenolic compounds in the leaves (5.45 and 4.75 mg GAE/g dry matter) than in the flowers (4.75 mg GAE/g dry matter) of *S. nipponica* with methanol extraction. Similar results have been reported by Boucheffa et al. [25] on *Pistacia lentiscus* leaves.

3.3. Antioxidant activity

The antioxidant potentials of the extracts were determined with DPPH radical scavenging activity. The DPPH is a free radical, stable at room temperature, and the most frequently used for determining antioxidant activity. The DPPH method is simple and based on only the reaction between the DPPH radical and the antioxidant [26]. Fig. 1 shows the DPPH radical scavenging activities of the extracts at varying concentrations. All the concentrations tested displayed antioxidant activities, and they increased significantly with concentrations up to 3.125 mg/mL ($P < 0.05$). However, at 6.25 mg/mL, there was no significant increase in the ethanol extracts ($P > 0.05$). Antioxidant activities of ethanol and methanol extracts of the flowers and the leaves at the lowest concentration (0.156 mg/mL) were determined as 12,3 ± 0.79, 21.6 ± 1.80, 29.4 ± 4.30 and 30.0 ± 0.67 %, while they were 66.43 ± 0.11, 69.76 ± 0.34, 65.71 ± 0.90 and 69.29 ± 2.58 % at the highest concentration (6.25 mg/mL), respectively. The antioxidant activity of the leaves was significantly higher than the flowers at 0.156 mg/mL concentration ($P < 0.05$). The DPPH results also indicated that methanol was more efficient

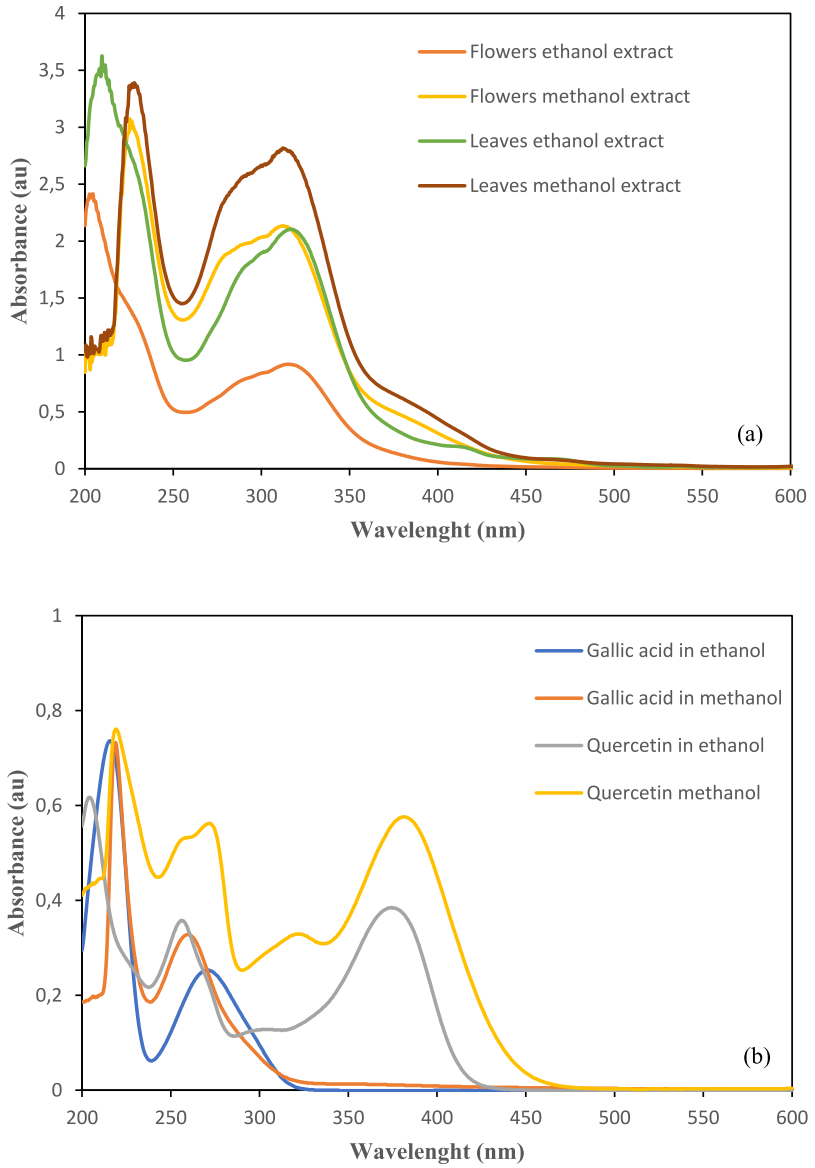

Fig. 2. Absorption spectra of the ethanol and methanol extracts of *S. japonica* var. *fortunei* leaves and flowers (a), Spectra of gallic acid and quercetin standards in ethanol and methanol (b).

than ethanol for extraction as with the total phenolic contents. IC_{50} is the concentration of compounds or extracts with antioxidant activity that can inhibit free radicals (DPPH) by 50 % and is derived from a linear regression equation that indicates the relationship between % DPPH activity and the concentration of compounds or extracts. The smaller the IC_{50} values, the better the antioxidant activity of the compound or extract [27]. In this study, IC_{50} values of ethanol and methanol extracts of the flowers and the leaves were estimated as 0.57 ± 0.06, 0.48 ± 0.01, 0.43 ± 0.01, and 0.45 ± 0.00 mg/mL, respectively. The extracts from the leaves had lower IC_{50} values than those from flowers, which correlated with the total phenolic contents of the same samples. It is known that the antioxidant activity of plant extracts is influenced by the phenolic contents, especially flavonoid compounds [28,29]. IC_{50} values of *S. japonica* var. *fortunei* were in accordance with the IC_{50} value of *S. prunifolia* which was reported as 320 μg/mL [30]. On the other hand, IC_{50} values of *S. japonica* var. *fortunei* were reasonably lower than those obtained from *S. media* with a different solvent, which was between 4.80 and 14.25 mg/mL [24].

3.4. Identification of the bioactive compounds

3.4.1. UV-VIS spectra of the extracts of S. japonica var. fortunei

UV-VIS spectra of the extracts were determined to confirm the presence of phenolic compounds, which absorb light in the UV

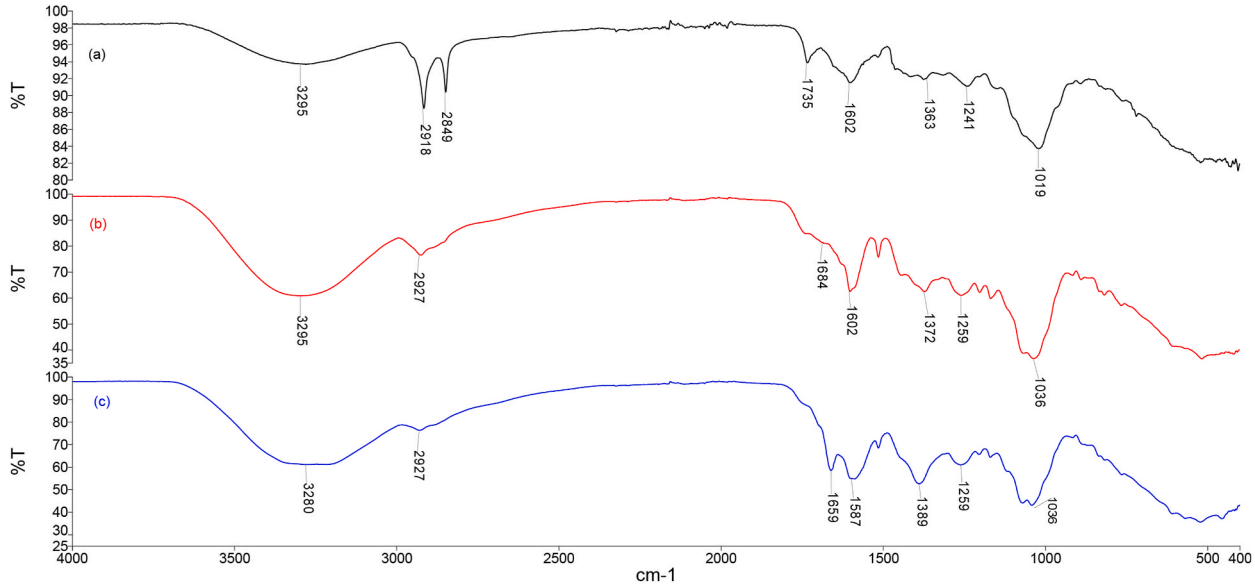

Fig. 3. FTIR spectra of the raw *S. japonica* var. *fortunei* leaves (a), ethanol (b), and methanol extracts (c) of the leaves.

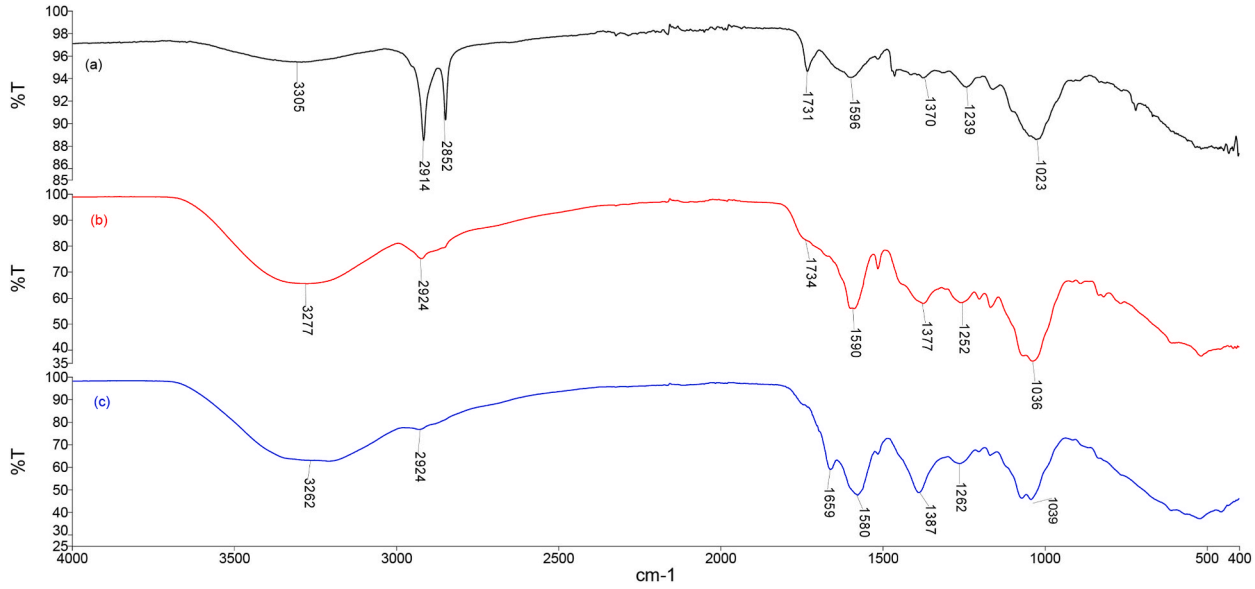

Fig. 4. FTIR spectra of the raw *S. japonica* var. *fortunei* flowers (a), ethanol (b), and methanol extracts (c) of the flowers.

region due to the n–π* electronic transition of aromatic moieties and chromophores [1]. Thus, absorption peaks in the UV–VIS region indicate the presence of alkaloids, flavonoids, phenolic acids, and tannins in plant extracts [2]. Absorption spectra of the ethanol and methanol extracts of *S. japonica* var. *fortunei* leaves and flowers are given in Fig. 2a. In general, three distinct absorption peaks were detected in all samples. The first peak was at 206–230 nm which differed based on the solvent used. The peaks were obtained at shorter wavelengths with the ethanol extracts. The second and third peaks were adjacent, of which one was detected at 270–293 nm, and the other at 314–320 nm. A slight increase in absorbance has also been observed at 384–422 nm. To compare, gallic acid and quercetin spectra were also determined in ethanol and methanol (Fig. 2b). Gallic acid displayed two peaks at 216–219 nm and 260–271 nm, while quercetin exhibited four peaks at around 204–219, 256–272, 305–322, and 374–382 nm. The peaks in the UV region (210–320 nm) confirmed the presence of phenolic compounds in *S. japonica* var. *fortunei* flowers and leaves extracts. Similar UV-VIS spectra were reported by the extract of bearberry leaves, *Dillenia pentagyna*, *Physalis* [1,2,31]. It was shown that plant extracts having higher antioxidant capacities generally have higher absorbance peaks at 280 and 358 nm [1]. Flavonoids act as UV filters to protect plants from biotic and abiotic stresses [32]. All flavonoids have an absorption peak at 240–290 nm owing to conjugated ring A, and the

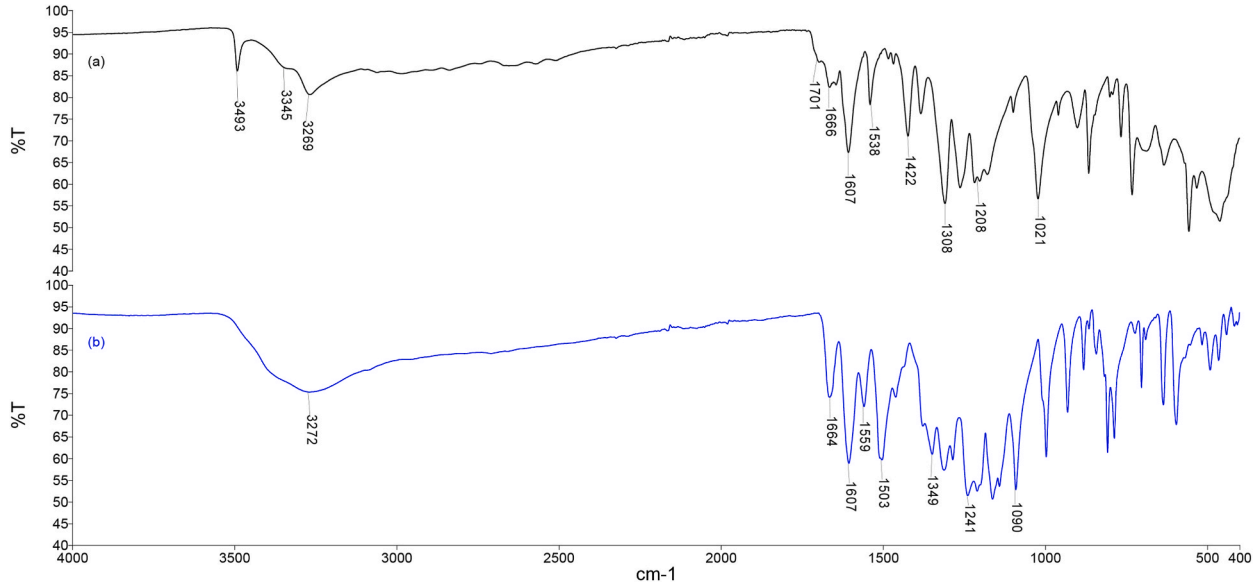

Fig. 5. FTIR spectra of gallic acid (a), and quercetin (b).

flavonoids having conjugated rings B and C exhibit an absorption peak at 300–500 nm [33].

3.4.2. *FTIR spectra of the extracts of S. japonica var. fortunei*

Fourier transform infrared analyses of the extracted samples of *S. japonica* var. *fortunei* were performed to identify the functional groups associated with the phenolic compounds. In addition, FTIR spectra of the raw flower and leaf samples and standard gallic acid and quercetin were determined to compare with the extracts.

Fourier transform infrared spectra of the raw leaf powder and its ethanol and methanol extracts are given in Fig. 3a, b, and c, respectively. The characteristic peaks of broad O–H stretching vibration were observed at 3300-3200 cm^{-1} in all the samples attributed to phenolic compounds and alcohols [34,35]. Intense peaks at 2849 and 2916 cm^{-1} were detected in raw leaves representing the stretching vibration of the non-aromatic C–H group. However, they were very slight or disappear in the extracts. The carbonyl group (C = 0) stretching peaks appeared at 1735 cm^{-1}, 1684 cm^{-1}, and 1661 cm^{-1} in the raw leaves, ethanol, and methanol extracts, respectively. The peak at 1602 cm^{-1} indicated aromatic C═C group vibration in the raw leaves, and the vibration peaks appeared at 1602 and 1587 cm^{-1} in ethanol and methanol extracts, respectively. The region 1500-500 cm^{-1} refers to a fingerprint that is unique for a specific compound and provides information about the molecular structure [2,36]. Almost similar spectra were obtained with ethanol and methanol extracts from the leaves in the fingerprint region, while it was slightly different in the raw leaf sample. The peaks at 1019 and 1241 cm^{-1} in that region indicate the stretching vibration of the C–O group. The peaks at around 1300 cm^{-1} suggested the bending vibration of the O–H group. The peaks at around 1200 and 1000 cm^{-1} were assigned to the stretching vibration of the C–*O*–C group [2,37].

Fourier transform infrared spectra of raw flowers and their ethanol and methanol extracts that were shown in Fig. 4a, b, and c, respectively, were almost identical to the leaf samples. Hence it indicated the presence of the same functional groups in both leaves and flowers. However, the intensity of the O–H stretching vibration (3300-3200 cm^{-1}) in the raw leaves was higher than in the raw flower. On the contrary, the peaks related to the raw flowers at 2917 and 2849 cm^{-1} were more intense than those of the raw leaves.

In the gallic acid spectrum (Fig. 5a), the peaks at 3493, 3345, and 3267 cm^{-1} are characteristics of gallic acid and were attributed to different O–H groups in the molecule [38]. The stretching vibration peak of the carbonyl group of gallic acid was observed at 1701 cm^{-1}. The peaks at 1608 and 1540 cm^{-1} belonged to the stretching vibrations of C–C bonds in the aromatic ring in the molecule. The 1300–1000 cm^{-1} peaks could be attributed to the stretching vibration of C–O and the bending vibration of O–H bonds of gallic acid [39]. In the quercetin FTIR spectrum (Fig. 5b), the stretching and bending vibrations of the O–H group appeared at 3272 and 1349 cm^{-1}, respectively. The peak at 1664 cm^{-1} was assigned to the C═O group. The aromatic C═C and C–C group's vibration peaks were observed at 1608 and 1559 cm^{-1}, respectively. The peaks at 1241 and 1090 cm^{-1} were assigned to C–O group vibration [2].

3.4.3. *LC-MS analysis of the flavonoids*

Profiles of metabolites from the methanol extracts of the leaves and the flowers of *S. japonica* var. *fortunei* were determined with LC-MS applying both positive and negative ionization. LC-MS chromatograms regarding the negative ionization modes of flower and leaf extracts are given in Fig. 6a and b, respectively. Almost identical peaks were observed in both samples, indicating their chemical contents were similar. A total of 36 compounds were observed in the extracts at negative ion mode. Epigallocatechin, chlorogenic acid, 3,5,7,3′,4′-pentahydroxy-6,8-dimethoxyflavone, isoquercetin, and avicularin were only detected in the leaf extracts, while agestricin D was found only in the flower extract. The rest of the compounds were present in both extracts. Fig. 6c and d shows the LC-MS

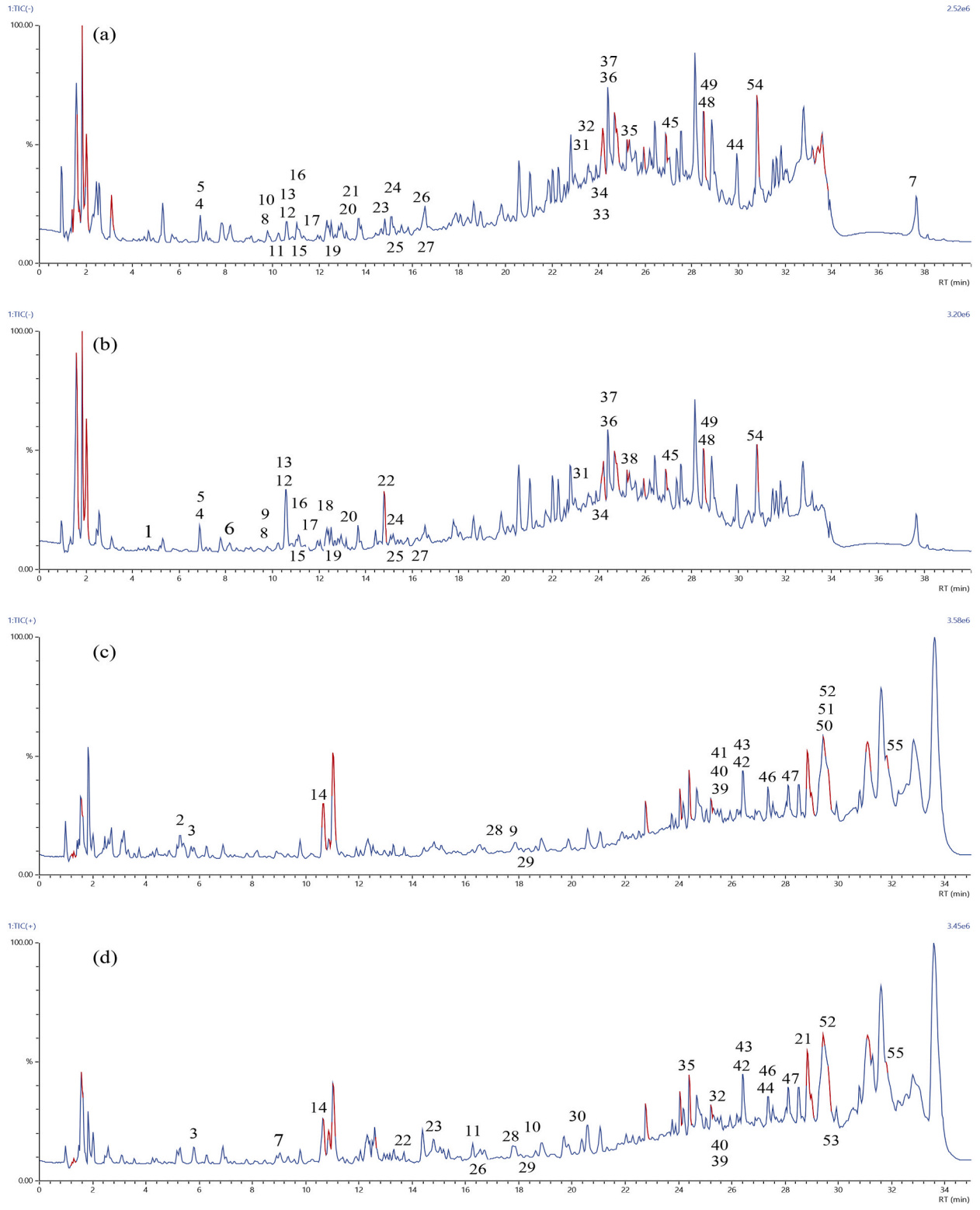

Fig. 6. LC-MS chromatograms of *S. japonica* var. *fortunei* extracts. (a) negative ion chromatogram of the flower extract, (b) negative ion chromatogram of the leaf extract, (c) positive ion chromatogram of the flower extract, (d) positive ion chromatogram of the leaf extract.

Table 3
Profile of polyphenols detected in the methanol extract of *S. japonica* var. *fortunei* by LC-MS.

Peak No	Retention time (min)	Compound	ESI mode	MW (amu)	Leaf and/or Flower
1	4.67	Epigallocatechin	–	306.27	L
2	5.70	Casticin	+	374.00	F
3	5.79	Catechin 7-*O*-α-L-rhamnopyranoside	+	436.41	F/L
4	6.87	Catechin-7-α-L-arabinofuranoside	–	422.38	F/L
5	6.87	Cichoric acid	–	474.37	F/L
6	8.12	Chlorogenic acid	–	354.31	L
7	37.63/9.33	Syringic acid	−/+	198.17	F/L
8	9.76	Quercetin 3,5-*O*-diglucoside	–	626.52	F/L
9	9.76/17.80	3,5,7,3′,4′-pentahydroxy-6,8-dimethoxyflavone	−/+	362.00	L
10	9.80/18.91	Centaureidin	+	360.00	F/L
11	10.22/16.53	Coumaroyl-quinic acid	−/+	338.31	F/L
12	10.57	Quinic acid	–	192.17	F/L
13	10.57	4-*O*-caffeoylquinic	–	354.31	F/L
14	10.60	3′-hydroxy-5,6,7,4′- tetramethoxyflavone	+	358.00	F/L
15	11.04	3′,4′,5,7-tetrahydroxy-3,6,8-trimethoxyflavone	+	376.00	F/L
16	11.11	5-*O*-feruloylquinic acid	–	368.34	F/L
17	11.92	Caffeic acid	–	180.16	F/L
18	12.33	Isorhamnetin 3-*O*-rhamnoside	–	462.40	F/L
19	12.58	4′,5,6,7-tetramethoxyflavone	–	342.34	F/L
20	13.67	Luteolin-4′-*O*-rutinoside	–	596.53	F/L
21	13.69/29.43	Kaempferol-3-*O*-rutinoside	−/+	594.00	F/L
22	15.06/13.70	Isoquercetin	−/+	464.00	L
23	14.42/14.80	7-hydroxy-3,3′,4′,5,6,8-hexamethoxyflavone	−/+	418.00	F/L
24	15.06	Hyperoside	–	464.38	F/L
25	15.49	1,5-dicaffeoylquinic acid	–	516.45	F/L
26	16.68/16.69	Quercetin 3-*O*- β -D-2-glucosyl-rutinoside	−/+	772.66	F/L
27	16.69	Narcissin	–	624.54	F/L
28	17.80	5,3′,5′-trihydroxy-3,6,7,4′-tetramethoxyflavone	+	390.00	F/L
29	18.10	Amentoflavone	+	538.00	F/L
30	20.35	Kaempferitrin	+	578.52	L
31	23.56	Catechin	–	290.27	F/L
32	23.88/25.92	3′,4′,5,6,7-pentahydroxy-3-methoxyflavone	−/+	332.00	F/L
33	24.19	Agestricin D	–	346.33	F
34	24.19	Nevadensin	–	344.32	F/L
35	25.58/24.70	Ombuin	−/+	330.00	F/L
36	24.72	Rhamnazin	–	330.29	F/L
37	24.72	Subscandenin	–	316.31	F/L
38	25.23	Avicularin	–	434.35	L
39	25.92	Marionol	+	388.37	F/L
40	25.92	4′-hydroxy-5,6,7,8,3′-pentamethoxyflavone	+	388.00	F/L
41	25.92	Salicylic acid	+	138.12	F
42	26.42	Apigenin 5,7-dimetyhyl ether	+	298.00	F/L
43	26.44	Isorhamnetin	+	316.00	F/L
44	30.81/27.35	Diosmetin	−/+	300.00	F/L
45	27.35	Padmatin	–	318.28	F/L
46	27.35	Homoeriodictyol	+	302.28	F/L
47	28.49	Persicogenin	+	316.31	F/L
48	28.50	Cirsimaritin	–	314,29	F/L
49	28.85	Kumatakenin	–	314.00	F/L
50	29.40	Quercetin	+	302.00	F
51	29.43	Aromadendrin-4′-methyl ether	+	302.28	F
52	29.45	Tiliroside	+	594.52	F/L
53	29.93	Gossypetin	+	318.00	L
54	30.81	Rhamnocitrin	–	300,26	F/L
55	32.06	Rhamnetin-3-*O*-β-D-glucopyranoside	+	478.40	F/L

chromatograms of flower and leaf extracts, respectively, at positive ion mode. Among 30 compounds determined at positive ion mode, casticin, salicylic acid, quercetin, and aromadendrin-4′-methylether were detected in the flower extracts. On the other hand, 3,5,7,3′,4′-pentahydroxy-6,8-dimethoxyflavone, isoquercetin, kaempferitrin, and gossypetin were found in the leaf extracts. All of the phenolic compounds determined from LC-MS chromatograms are elucidated in Table 3, and their ion chromatograms, along with the chemical structures, are given in Supplementary File (S1).

Among the various phenolic compounds detected in the leaves of *S. japonica* var. *fortunei*, some specific ones have been shown to be crucial for human health. Casticin, which was only detected in the flower sample, is a crucial flavonoid known for its analgesic, anti-inflammatory, antiasthmatic, and antiangiogenic characteristics. Recently its antineoplastic properties were also reported as a potent natural anticancer treatment agent. Casticin was also determined in *Vitex trifolia, V. negundo, Psiada trinervia, Daphne genkw* and *Pluchea quitoc* [40]. The presence of casticin in the flowers of *S. japonica* var. *fortune* was ascertained for the first time in this study.

Aromadendrin-4′-methylether has been shown to possess antimicrobial and antioxidant activities [41]. Quercetin is the most abundant flavonoid that was also detected in the flower extract, while it was in the form of isoquercetin in the leaf extract.

Ombuin is known for its pancreatic α-amylase inhibitory effect and was offered as a therapeutic candidate for treating diabetes mellitus [42]. Centaureidin can activate Rho, which leads to cytoskeletal reorganization and dendrite retraction and decreases melanosome transfer from melanocytes [43]. Cichoric acid is widely used in drugs, nutritional supplements, and functional foods due to its promising pharmacological effects in regulating glucose and lipid metabolism [44]. Salicylic acid is used for peeling acne due to the exfoliating ability of the stratum corneum [45]. Hyperocyte exhibits various pharmacological effects, such as protecting blood vessels, regulating the digestive system, and protecting against oxidation, aging, and cancer [46]. Cirsimaritin has been reported to possess multiple biological effects, including antimicrobial, anti-inflammatory, and antiproliferative properties. It can also be used as an antidiabetic [47]. Isorhamnetin has an extensive pharmacological effects such as cardiovascular protection, anti-inflammation, anti-tumor, anti-oxidation, anti-bacterial, and anti-virus. It also can potentially prevent neurodegenerative diseases such as Alzheimer's disease [48]. Amentoflavone has been reported to display numerous biological activities, including anti-inflammatory, antibacterial, antifungal, antivirus, anti-oxidative, antiangiogenesis, neuroprotection, osteogenesis, anti-arthritis, radioprotection, antidiabetic, and antidepressant [49]. Diosmetin is an important flavonoid having anti-cancer, anti-inflammatory, antioxidant, and oestrogenic activities [50]. Anticancer activities of persicogenin, and homoeriodictyol were reported by Saquib et al. [51].

4. Conclusion

Phenolics and flavonoids in the leaves and flowers of *S. japonica* var. *fortunei* were investigated both qualitatively and quantitatively for the first time in this study. Notable amounts of total phenolic and flavonoids were detected in the leaves and flowers. However, the plant leaves were richer in bioactive compounds than the flowers. Methanol was the better extraction solvent. Both the leaves and the flowers had antioxidant activities. The bioactive compounds in the extracts were also identified and confirmed with FTIR and UV-VIS spectra analyses. LC-MC analyses showed that the plant contained valuable flavonoids such as casticin, ombuin, centaureidin, isorhamnetin, diosmetin, persicogenin, homoeriodictyol, and amentoflavone that have outstanding therapeutic properties. Hence, this study revealed that *S. japonica* var. *fortunei* is a promising source of some specific flavonoids and can be a potential candidate for pharmacological and therapeutic applications.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Semra Yılmazer Keskin: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ayşe Avcı:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Haka Fajriana Febda Kurnia:** Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Can Serkan Keskin (Sakarya University, Chemistry Department) for helping in FTIR analysis.

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25496.

References

[1] X.C. Song, E. Canellas, E. Asensio, C. Nerín, Predicting the antioxidant capacity and total phenolic content of bearberry leaves by data fusion of UV–Vis spectroscopy and UHPLC/Q-TOF-MS, Talanta 213 (2020) 120831, https://doi.org/10.1016/j.talanta.2020.120831.

[2] T.K. Patle, K. Shrivas, R. Kurrey, S. Upadhyay, R. Jangde, R. Chauhan, Phytochemical screening and determination of phenolics and flavonoids in *Dillenia pentagyna* using UV–vis and FTIR spectroscopy, Spectroc. Acta Pt. A-Molec. Biomolec. Spectr. 242 (2020) 118717, https://doi.org/10.1016/j.saa.2020.118717.

[3] S. Keskin, Y. Sirin, H.E. Cakir, G. Kaya, M. Keskin, Phenolic composition and antioxidant properties of *Spireaea nipponica*, International Journal of Scientific and Technological Research 5 (2) (2019) 87–92, https://doi.org/10.7176/JSTR/5-2-10.

[4] A. Egdhami, F. Sadeghi, Determination of total phenolic and flavonoids contents in methanolic and aqueous extract of *Achillea millefolium*, Org. Chem. J. 2 (2010) 81–84.
[5] S. Gharibi, B.E.S. Tabatabaei, G. Saeidi, S.A.H. Goli, M. Talebi, Total phenolic content and antioxidant activity of three Iranian endemic *Achillea* species, Ind. Crop. Prod. 50 (2013) 154–158, https://doi.org/10.1016/j.indcrop.2013.07.038.
[6] A.X. Cheng, X.J. Han, Y.F. Wu, H.X. Lou, The function and catalysis of 2-oxoglutarate-dependent oxygenases involved in plant flavonoid biosynthesis, Int. J. Mol. Sci. 15 (2014) 1080–1095, https://doi.org/10.3390/ijms15011080.
[7] Y. Ma, X.Y. Mao, L.J. Huang, Y.M. Fan, W. Gu, C. Yan, T. Huang, J.X. Zhang, C.M. Yuan, X.J. Hao, Diterpene alkaloids and diterpenes from *Spiraea japonica* and their anti-tobacco mosaic virus activity, Fitoterapia 109 (2016) 8–13, https://doi.org/10.1016/j.fitote.2015.11.019.
[8] M.H. Yean, J.S. Kim, S.S. Kang, Y.S. Kim, A new megastigmane glucoside and three new flavonoid glycosides from *Spiraea prunifolia* var. *simpliciflora*, Helv. Chim. Acta 97 (2014) 1123–1131.
[9] Q. Wang, M.M. Chen, X.F. Hu, R.H. Wang, Q.L. He, The complete chloroplast genome sequence of *Spiraea japonica* var. acuminate Franch. (Rosaceae), Mitochondrial DNA B 7 (1) (2022) 275–276, https://doi.org/10.1080/23802359.2022.2028590.
[10] Z. Zhao-Yang, S. Hang, G. Zhi-Jian, Karyomorphological study of the *Spiraea japonica* complex (Rosaceae), Brittonia 54 (3) (2002) 168–174.
[11] L. Li, Y.M. Shen, X.S. Yang, G.Y. Zuo, Z.Q. Shen, Z.H. Chen, X.J. Hao, Antiplatelet aggregation activity of diterpene alkaloids from *Spiraea japonica*, Eur. J. Pharmacol. 449 (2002) 23–28.
[12] L.M. Fan, H.P. He, Y.M. Shen, X.J. Hao, Two new diterpenoid alkaloids from *Spiraea japonica* L. f. var. *fortunei* (Planchon) Rehd, J. Integr. Plant Biol. 47 (1) (2005) 120–123.
[13] V.A. Kostikova, N.V. Petrova, Phytoconstituents and bioactivity of plants of the genus *Spiraea* L. (Rosaceae): a review, Int. J. Mol. Sci. 22 (2021) 11163, https://doi.org/10.3390/ijms222011163.
[14] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.
[15] A. Avcı, A. Kamiloğlu, S. Dönmez, Efficient production of acetone butanol ethanol from sole fresh and rotten potatoes by various *Clostridium* strains, Biomass Conv. Bioref. 13 (2021) 4161–4169, https://doi.org/10.1007/s13399-021-01584-w.
[16] S. Kamtekar, V. Keer, V. Patil, Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation, J. Appl. Pharm. Sci. 4 (9) (2014) 61–65, https://doi.org/10.7324/JAPS.2014.40911.
[17] W. Brand-Williams, M. Cuvelier, C. Berset, Use of a free-radical method to evaluate antioxidant activity, Food Sci. Technol.-Lebensm.-Wiss. Technol. 28 (1) (1995) 25–30.
[18] S. Sen, B. De, N. Devanna, R. Chakraborty, Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of *Meyna spinosa* Roxb., an Indian medicinal plant, Chin. J. Nat.0 Med. 11 (2) (2013) 0149−0157.
[19] A. Görgüç, P. Özer, F.M. Yılmaz, Simultaneous effect of vacuum and ultrasound assisted enzymatic extraction on the recovery of plant protein and bioactive compounds from sesame bran, J. Food Compos. Anal. 87 (2020) 103424.
[20] S. Mazzuttia, C.A.S. Riehlb, E. Ibānezc, S.R.S. Ferreiraa, Green-based methods to obtain bioactive extracts from *Plantago major* and *Plantago lanceolate*, J. Supercrit. Fluids 119 (2017) 211–220, https://doi.org/10.1016/j.supflu.2016.09.018.
[21] O. Alhajali, A. Ali-Nizam, Phytochemical screening and antibacterial activity of *Pistacia atlantica* and *Pinus canariensis* leaf extracts, J. Turkish Chem. Soc. 8 (2) (2021) 403–418, https://doi.org/10.18596/jotcsa.836074.
[22] A.A. Mostafa, Al Askar, A.A. Khalid S, K.S. Almaary, T.M. Dawoud, N. Essam, E.N. Sholkamy, M.M. Bakri, Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, Saudi J. Biol. Sci. 25 (2018) 361–366, https://doi.org/10.1016/j.sjbs.2017.02.004.
[23] F.D. Gonelimali, J. Lin, W. Miao, J. Xuan, F. Charles, M. Chen, S.R. Hatab, Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms, Front. Microbiol. 9 (2018) 1–9, https://doi.org/10.3389/fmicb.2018.01639.
[24] T.I. Shirshova, K.V. Bezmaternyk, I.V. Beshlei, A.N. Smirnova, O.N. Oktyabr'skii, Antioxidant properties of extracts of leaves and inflorescences of *Spiraea media* Franz Schmidt from the flora of Komi Republic, Pharm. Chem. J. 54 (6) (2020) 45–48, https://doi.org/10.1007/s11094-020-02246-3.
[25] S. Boucheffa, W. Sobhi, A. Attoui, S. Selli, H. Kelebek, A. Semmeq, Y. Benguerba, Effect of the main constituents of Pistacia lentiscus leaves against the DPPH radical and xanthine oxidase: experimental and theoretical study, J. Biomol. Struct. Dyn. 40 (20) (2022) 9870–9884.
[26] K. Sirivibulkovit, S. Nouanthavong, Y. Sameenoi, Paper-based DPPH assay for antioxidant activity analysis, Anal. Sci. 34 (7) (2018) 795–800.
[27] Y. Zou, Y. Lu, D. Wei, Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. in vitro, J. Agric. Food Chem. 52 (16) (2004) 5032–5039, https://doi.org/10.1016/j.lfs.2004.05.001.
[28] N. Huda-Faujan, Z.A. Rahim, M.M. Rehan, F.B.H. Ahmad, Comparative analysis of phenolic content and antioxidative activities of eight Malaysian traditional vegetables, Malaysian J. Anal. Sci. 19 (3) (2015) 611–624.
[29] G.A. Gruyal, R.R.D. Rosario, Phytochemical profiles and quantifications of flavonoid contents of selected herbs in cantilan, surigao del sur Philippines, SDSSU Int. Multidiscip. Res. J. 1 (2) (2013) 126–133.
[30] M.O. Sim, H.J. Lee, J.H. Jang, H.E. Lee, H.K. Jung, T.M. Kim, J.H. No, J. Jung, D.E. Jung, H.W. Cho, Anti-inflammatory and antioxidant effects of *Spiraea prunifolia* Sieb. et Zucc. var. *simpliciflora* Nakai in RAW 264.7 Cells, Korean J. Plant Res. 30 (4) (2017) 335–342, https://doi.org/10.7732/kjpr.2017.30.4.335.
[31] C. Karpagasundari, S. Kulothungan, Analysis of bioactive compounds in *Physalis minima* leaves using GC MS, HPLC, UV-VIS and FTIR techniques, Int. J. Pharmacogn. Phytochem. Res. 3 (4) (2014) 196–201.
[32] A.N. Panche, A.D. Diwan, S.R. Chandra, Flavonoids: an overview, J. Nutr. Sci. 5 (47) (2016) 1–15, https://doi.org/10.1017/jns.2016.41.
[33] M. Vihakas, Flavonoids and Other Phenolic Compounds: Characterization and Interactions with Lepidopteran and Sawfly Larvae, University of Turku Department of Chemistry/Faculty of Mathematics and Natural Sciences, 2014, pp. 1–78. Doctoral thesis.
[34] D.S.F. Grasel, M.F. Ferrão, C.R. Wolf, Development of methodology for identification the nature of the polyphenolic extracts by FTIR associated with multivariate analysis, Spectroc. Acta Pt. A-Molec. Biomolec. Spectr. 153 (2016) 94–101, https://doi.org/10.1016/j.saa.2015.08.020.
[35] N. Sahu, J. Saxena, Phytochemical analysis of *Bougainvillea Glabra Choisy* by FTIR and UV-VIS spectroscopic analysis, Int. J. Pharm. Sci. Rev. Res 21 (1) (2013) 196–198.
[36] K. Kalaichelvi, S.M. Dhivya, Screening of phytoconstituents, UV-VIS spectrum and FTIR analysis of *Micrococca mercurialis* (L.) Benth, Int. J. Herb. Med. 5 (6) (2017) 40–44.
[37] S.S. Kumar, P. Manoj, P. Giridhar, Fourier transform infrared spectroscopy (FTIR) analysis, chlorophyll content and antioxidant properties of native and defatted foliage of green leafy vegetables, J. Food Sci. Technol. 52 (12) (2015) 8131–8139, https://doi.org/10.1007/s13197-015-1959-0.
[38] P.L. Lam, K.K.H. Lee, S.H.L. Kok, G.Y.M. Cheng, X.M. Tao, D.K.P. Hau, M.C.W. Yuen, K.H. Lam, R. Gambari, C.H. Chui, R.S.M. Wong, Development of formaldehyde-free agar/gelatin microcapsules containing berberine HCl and gallic acid and their topical and oral applications, Soft Matter 8 (18) (2012) 5027–5037, https://doi.org/10.1039/C2SM07236J.
[39] S. Naz, A.R. Khaskheli, A. Aljabour, H. Kara, F.N. Talpur, S.T.H. Sherazi, A.A. Khaskheli, S. Jawaid, Synthesis of highly stable cobalt nanomaterial using gallic acid and its application in catalysis, Adv. Chem. (2014) 1–6, https://doi.org/10.1155/2014/686925.
[40] S. Ramchandani, I. Naz, J.H. Lee, M.R. Khan, K.S. Ahn, An overview of the potential antineoplastic effects of casticin, Molecules 25 (6) (2020) 1287.
[41] B.I. Nwiloh, C.C. Monago-Ighorodje, G.N. Onwubiko, Analyses of bioactive compounds in fiddleheads of Pteridium aquilinum L. Kuhn collected from Khana, Southern Nigeria, using gas chromatography-flame ionization detector, J. Pharmacogn. Phytochem. 9 (2) (2020) 1079–1086.
[42] B. Kikiowo, J.A. Ogunleye, O. Iwaloye, T.T. Ijatuyi, Therapeutic potential of Chromolaena odorata phyto-constituents against human pancreatic α-amylase, J. Biomol. Struct. Dyn. 40 (4) (2022) 1801–1812.
[43] Y. Ito, A. Kanamaru, A. Tada, Centaureidin promotes dendrite retraction of melanocytes by activating Rho, Biochimica et Biophysica Acta (BBA)-General Subjects 1760 (3) (2006) 487–494.
[44] M. Yang, C. Wu, T. Zhang, L. Shi, J. Li, H. Liang, F. Li, Chicoric acid: natural occurrence, chemical synthesis, biosynthesis, and their bioactive effects, Front. Chem. (10) (2022) 1–16.
[45] T. Arif, Salicylic acid as a peeling agent: a comprehensive review, Clin. Cosmet. Invest. Dermatol. (2015) 455–461.

[46] S. Xu, S. Chen, W. Xia, H. Sui, X. Fu, Hyperoside: a review of its structure, synthesis, pharmacology, pharmacokinetics and toxicity, Molecules 27 (9) (2022) 3009.
[47] T. Benali, I. Jaouadi, R. Ghchime, N. El Omari, K. Harbou, K. Hammani, A. Bouyahya, The Current state of knowledge in biological properties of Cirsimaritin, Antioxidants 11 (9) (2022) 1842.
[48] G. Gong, Y. Guan, Z. Zhang, K. Rahman, S. Wang, S. Zhou, X. Luan, H. Zhang, Isorhamnetin: a review of pharmacological effects, Biomed. Pharmacother. (2020) 1–15.
[49] Y. Xiong, P. Zhang, R.D. Warner, S. Shen, S. Johnson, Z. Fang, Comprehensive profiling of phenolic compounds by HPLC-DAD-ESI-QTOF-MS/MS to reveal their location and form of presence in different sorghum grain genotypes, Food Res. Int. 137 (2020) 109671.
[50] K. Patel, M. Gadewar, V. Tahilyani, D.K. Patel, A review on pharmacological and analytical aspects of diosmetin: a concise report, Chin. J. Integr. Med. 19 (2013) 792–800.
[51] Q. Saquib, S. Ahmed, M.S. Ahmad, A.J. Al-Rehaily, M.A. Siddiqui, M. Faisal, A.A. Al-Khedhairy, Anticancer efficacies of persicogenin and homoeriodictyol isolated from Rhus retinorrhoea, Process Biochemistry 95 (2020) 186–196.