Valorization of Fig (*Ficus carica* Linn.) Waste Leaves: HPLC-QTOF-MS/MS-DPPH System For Online Screening And Identification of Antioxidant Compounds

Chunying Li

Northeast Forestry University

Meiting Yu

Northeast Forestry University

Shen Li

Northeast Forestry University

Xue Yang

Northeast Forestry University

Bin Qiao

Northeast Forestry University

Sen Shi

Northeast Forestry University

Chunjian Zhao (✉ zcjsj@163.com)

Northeast Forestry University <https://orcid.org/0000-0003-2827-1999>

Yujie Fu

Northeast Forestry University

Research Article

Keywords: *Ficus carica* Linn, waste leaves, Antioxidant, HPLC-QTOF-MS/MS-DPPH, Flavonoids, Polyphenols

Posted Date: September 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-804140/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Plants on November 21st, 2021. See the published version at <https://doi.org/10.3390/plants10112532>.

Abstract

Fig (*Ficus carica* Linn.) leaves are produced each year and often thrown away as wastes, resulting in a waste of resources. Fig waste leaves are rich in flavonoids, which have strong antioxidant activity, however, the antioxidant components are not clear. So as to take full advantage of fig waste leaves, antioxidant capacity of different extracts (petroleum ether, ethyl acetate and water) was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) and ferric-ion reducing antioxidant power (FRAP) methods. The results showed that flavonoids in ethyl acetate extraction had the highest content, 83.92 ± 0.01 mg/g, and maximum DPPH scavenging activity (IC_{50} 0.54 mg/mL), highest ABTS scavenging rate (80.28%), FRAP (3.46 mmol/g). Furthermore, HPLC-QTOF-MS/MS-DPPH method was developed for the first time to identify 11 flavonoids in fig waste leaves. This rapid and efficient method can not only be used for screening the antioxidant components in fig waste leaves, but also can be combined with mass spectrometry to identify the compounds with antioxidant capacity. There are three flavonoids with significant antioxidant capacity, which are 3-O-(rhamnopyranosyl-glucopyranosyl)-7-O-(glucopyranosyl)-quercetin, isoschaftoside and rutin. The results confirmed that fig waste leaves contain a variety of antioxidant components, which contributed to increase the value of fig waste leaves as antioxidants.

Statement Of Novelty

A large number of fig leaves are produced each year and often thrown away as wastes, resulting in a waste of resources. However, fig leaves are rich in flavonoids and polyphenols, which have strong antioxidant activity. HPLC-QTOF-MS/MS-DPPH method was used for online screening and identification of components with antioxidant activity from fig waste leaves for the first time. Chemical structures were identified and the antioxidant capacity were measured. This study provided a scientific and theoretical basis for the development and utilization of waste biomass from fig leaves.

Introduction

Fig (*Ficus carica* Linn.) as one of the earliest cultivated fruit trees, belongs to the mulberry family (Moraceae). Figs are native to the Mediterranean coast, from Turkey to Afghanistan [1], mainly grow in some tropical and temperate regions, belonging to the subtropical larch family [2]. It was introduced into China from Persia in the Tang Dynasty. Because of its low requirements on soil conditions and strong roots, it was widely cultivated in the north and south, mainly distributed in Xinjiang, Fujian, Shandong and other places. Fig has not only a wide range of medicinal and nutritional value, but also has nourishing the stomach and clearing the intestines, reducing swelling and detoxification. It is often used in the treatment of anorexia, abdominal distension and abdominal pain, hemorrhoids and constipation, dyspepsia, insufficient milk, sore throat, cough and sputum [3, 4, 5].

To make the fig trees grow more vigorously, they are often been pruned. Therefore, a large number of abandoned branches and leaves will be produced every year, causing environmental pollution and waste

of resources. However, the fig leaves were found to contain flavonoids, sugars, pectin, tannins, vitamin C (Vc) and trace elements [6, 7]. Clinical studies had shown that fig leaves extract had anti-tumor, hypolipidemic, antioxidant, antibacterial, hypoglycemic and other activities [8, 9, 10]. Because the fig leaves contain a large number of flavonoids, they have a variety of pharmacological activities, which can prevent cardiovascular diseases, anti-osteoporosis, treatment of diarrhea, scavenging oxidative free radicals, lowering blood lipids, treatment of sore throat and immune regulation [11, 12, 13, 14, 15, 16, 17]. It is reported that higher amount of flavonoids have been found in fig leaves [18], including rutin, quercetin, anthocyanin and so on. Anthocyanins enable to promote the apoptosis of cancer cells, slow down the proliferation of cancer cells and prevent the metastasis of cancer cells [19]. At the same time, it can effectively relieve visual fatigue and slow down the development of diabetic cataract [20]. Quercetin and rutin have protective effects on ischemic brain injury [21]. Flavonoids not only regulate the cardiovascular system by reducing capillary permeability, lipid and blood pressure, but also play an important role in anti-inflammatory and anti-immune activity by affecting cell mitosis, cell-cell interaction and cell secretion [22, 23]. Therefore, making full use of fig waste leaves is an effective way to realize resource utilization.

1, 1-diphenyl-2-picrylhydrazyl (DPPH) is a very stable nitrogen-centered free radical that can trap other free radicals and has maximum absorption at 517 nm. It has a strong purple color and turns yellow after reacting with antioxidant species. This method was often used to evaluate the antioxidant activity of compounds or plant extracts. [24]. Nevertheless, in the evaluation of antioxidant activity of herbal extracts with complex components, the main drawback of this method is that it is impossible to know exactly which compounds in the complex mixture have antioxidant activity. Only after the separation and purification of a compound it can be known whether it has antioxidant activity. This method is time-consuming, laborious, and the results are not accurate [25]. Recently, HPLC-DPPH-DAD online screening of antioxidant systems is a fast and efficient method for exploring antioxidant compounds from natural resources [26, 27] and used for screening and identification of antioxidants in fruit wine, tea, plants and so on [28, 29, 30].

In this study, HPLC-DPPH was used to screen out the antioxidant active components in different extracts of fig waste leaves. In order to further study the antioxidant chemical components, HPLC-DPPH-MS/MS was applied to identify and analyze the antioxidant active components for the first time. By this method, 11 flavonoids were found in the fig waste leaves. Compared with the traditional methods for screening natural antioxidant active components, this method can screen and identify the antioxidant active components in fig waste leaves rapidly and efficiently, so as to make full use of the fig resources.

Materials And Methods

Chemicals and materials

Discarded fig leaves were picked from Chengshan Town, Rongcheng City, Shandong Province, China. And the fig leaves were dried at a shady place out of direct sunlight until the weight was remained the

same. The dried leaves were smashed and sieved through a 60-mesh sieve for further test. All chemicals were of analytical grade, unless stated otherwise and were purchased from Sigma Aldrich (St. Louis, MO, USA). Standard solutions were stored at 4 °C.

Instrumentation

An Agilent 6530 Accurate-Mass QTOF-MS system was connected with HPLC system via an Agilent Jet Stream electrospray (ESI) interface and an Agilent 1260 HPLC with diode array detector (DAD) (Agilent Technologies, Santa Clara, CA, USA). Kq-100e Ultrasonic cleaning instrument (Kunshan Ultrasonic Instrument Co., LTD.) was used.

Sample preparation

The fig leaves wastes were dried at the temperature of 60 °C and then crushed through a 60-mesh screen. 10 g of dry powder was weighed and put into a triangular flask, 70% ethanol was added at the ratio of 1:10 (w/v), 200 W, 40 °C, ultrasonic for 30 min, repeated twice, centrifugation at 6000 rpm for 10 min, filtrate was combined, concentration was performed to obtain the ethanol extract of fig leaves. An appropriate amount of distilled water was added to the ethanol extract to form a homogeneous suspension, which was extracted by adding petroleum ether and ethyl acetate in turn to be extracted by rotary evaporation at 60 °C to obtain the extracts.

Total polyphenol content

The total polyphenol contents in different solvent extracts were based on Folin-Ciocalteu method. The optimal reaction conditions of the system were as follows: test sample 0.1 mL, Folin-Ciocalteu reagent 0.1 mL and 60 g/L Na₂CO₃ solution 0.8 mL, with minor modifications [31]. Mixture was incubated at room temperature and protected from light for 10 min. Absorbance of total polyphenol at 765 nm was determined by UV-Visible spectrometer.

Total flavonoid content

The content of total flavonoids in fig waste leaves was determined by NaNO₂-Al(NO₃)₃ method [32]. Rutin was used as a standard. Content of total flavonoid was measured at 506 nm of wavelength.

Determination of antioxidant capacities

DPPH radical scavenging capacity

A proper amount of DPPH sample was weighted and anhydrous ethanol was added for ultrasonic dissolution to obtain DPPH solution with the concentration of 0.06 mg/mL. 0.15 mL of DPPH solution from the stock was mixed with 0.17 mL of diluted sample. The reaction was kept from light for 30 min at room temperature. The detection wavelength was 517 nm. Each group of experiments is parallel for three times, and the calculation formula is as follows:

$$W = \frac{1 - (A_m - A_n)}{A_k} \times 100$$

.....(1)

where W (%) represents DPPH radical scavenging rate. A_k , A_m and A_n represent absorbance of DPPH solution, absorbance of extracts of different concentrations reacted with DPPH solution and absorbance of different concentrations of extracts reacted with anhydrous ethanol.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) radical scavenging capacity

The mixture of 100 μ L ABTS solution and oxidant solution was added at the volume ratio of 1:1 and was kept away from light at room temperature for 16 h. The ABTS working solution was obtained by diluting the ABTS solution with anhydrous ethanol. 20 μ L of different concentrations of extract solution, different concentration of Vc solution and 180 μ L ABTS working solution were mixed evenly in a 96-well plate. The absorbance was measure at 734 nm, and the experiments were repeated three times in each group. The scavenging capacity of ABTS free radicals is calculated by the following formula (2):

$$W = \frac{1 - (A_m - A_n)}{A_k} \times 100$$

.....(2)

where W (%) refers to ABTS radical scavenging rate. A_k , A_m and A_n refer to absorbance of ABTS working solution, absorbance of ABTS after reacting with sample and absorbance of sample solution mixed with anhydrous ethanol.

Ferric-ion reducing antioxidant power (FRAP)

FRAP was determined according to the method of Jin et al. [33]. Take 150 μ L of appropriately diluted extract of fig waste leaves, add 3 mL of 2, 3, 5-triphenyl-2h-tetrazolium, chloride (TPTZ) working solution, which consists of acetic acid buffer (pH 3.6), 10 mmol/L TPTZ solution and 20 mmol/L FeCl_3 solution mixed in proportion (10:1:1) and reacted at 37 °C for 30 min. The detection wavelength was 593 nm.

Establishment of the HPLC-QTOF-MS/MS-DPPH system

HPLC-DAD-ESI-MS analysis

HPLC-ion trap mass spectrometry with DAD was used to determine the components of fig waste leaves extract. The mobile phase was cosisted of 0.1% formic acid solution (A) and acetonitrile (B). The gradient elution conditions were as follows: 0–6 min, 15% B; 6–30 min, 15%–45% B; 30–40 min, 45%–60% B. The column was kept at 30 °C and detected at 330 nm. An Agilent 6530 Accurate-Mass QTOF-MS system

was connected with HPLC system via an Agilent Jet Stream electrospray (ESI) interface (Agilent Technologies, Santa Clara, CA). The electrospray ion source is in negative ion mode, the scanning range of mass spectrometer is from m/z 100 to 1500, the ion source is set at 550 °C, the ion source voltage (IS) -4500 V, the atomization gas pressure 55 psi. And the air curtain gas (CUR) pressure is 35 psi. The de-clustering voltage (DP) of the first-level scanning and the focusing voltage (CE) is 100 V and 10 V, respectively. The secondary mass spectrometry scan uses Product Ion-IDA mode to collect mass spectrum data, and the CID energy is set at -20, -40 and -60 V, respectively. Before injection, the CDS pump is used to correct the mass axis, so that the error of the quality axis is less than 2 ppm.

Online HPLC-DPPH analysis

Antioxidants in the fig waste leaves extract were screened by online HPLC-DPPH. The flow chart of online HPLC-DPPH screening system was shown in figure 1. This method takes into account the negative peaks produced by the reaction of antioxidants with DPPH radical. Separation was achieved on Agilent 1260 HPLC (Agilent Technologies infinity, Santa Clara, CA, USA). All HPLC-DPPH separation steps were carried out on a C_{18} column (4.6 mm × 250 mm, 5 μ m, Waters Technologies, USA). The flow rate of HPLC-separated analytes and 50 μ g/mL DPPH solution was set at 0.7 mL/min and 0.5 mL/min, respectively. The column temperature was maintained at 35 °C, the detection wavelength was 330 nm, and inject volume was 10 μ L. The online HPLC-DPPH analysis conditions were the same as mentioned above. The sample was reacted with DPPH solution in PEEK tube (10 m0.25 mm), determined at 521 nm, the absorption of the compound with antioxidant activity was reduced by pairing with single electron of DPPH with a negative peak.

Results And Discussion

Determination of total polyphenol content

The total polyphenol content of different solvent extracts of fig leaves was shown in figure 2a. It was obvious that the ethyl acetate extract showed the highest content of polyphenol compounds, which was (1.72±0.01) mg/g. In contrast, the content of polyphenol compounds measured in water and petroleum ether extracts was significantly lower, which might be related to the low solubility of polyphenol in petroleum ether and water.

Determination of total flavonoids content

The total flavonoids content of different solvent extracts of fig leaves was shown in figure 2b. The content of total flavonoids in the extracts was mainly related to different extraction solvents. The content of total flavonoids in the extracts of different solvents was determined. Total flavonoids content in the ethyl acetate part was the highest, which was (83.92±0.01) mg/g, and that in the petroleum ether part was the lowest, which was (18.71±0.11) mg/g.

Antioxidant capacity

DPPH, ABTS and FRAP were used to evaluate the antioxidant capacity of extracts from fig waste leaves. All the extracts of fig leaves with three different solvents all had the ability to scavenge DPPH free radicals, and with the increase of extract concentration, the DPPH scavenging rate gradually increased. The scavenging ability of ethyl acetate extract varied greatly with concentration. As is known, the lower the IC₅₀ value, the higher the antioxidant activity of the antioxidants. The IC₅₀ value of ethyl acetate extract was 0.54 mg/mL, which did not exceed the scavenging ability of Vc on DPPH free radical, nevertheless, the difference was not significant ($P>0.05$). It can be seen from figure 3a that the scavenging ability of three extracts on DPPH free radical decreased in the following order: ethyl acetate phase > water phase > petroleum ether phase. This may be related to the fact that the ethyl acetate extract contained more flavonoids and polyphenol compounds.

It can be seen from figure 3b that each fig leaves extract had the ability of scavenging ABTS free radicals, which was similar to that of DPPH free radical. The changing trend of sample scavenging ability was the same as that of sample concentration. The ethyl acetate extract had the strongest scavenging rate at different concentration of three extracts and the scavenging rate was 80.28% at the concentration of 2.5 mg/mL. The ABTS free radical scavenging activity decreased in the following order: ethyl acetate phase > water phase > petroleum ether phase, which also proved that the flavonoids of fig leaves had strong antioxidant activity. Flavonoids and polyphenols mostly have phenolic hydroxyl groups, which show moderate polarity and can bind with free radicals, showing strong antioxidant activity.

When the total antioxidant capacity was measured by FRAP method, the FRAP value was represented by the concentration of FeSO₄ solution. The higher the concentration of extracts, the stronger the antioxidant activity of the substance. The total antioxidant capacity was positively correlated with the concentration of extracts. Among the three extracts, ethyl acetate phase showed the highest FRAP value, 3.46 mmol/g and the strongest reducing ability to ferric ion, which was significantly higher than that of water and petroleum ether phase. The ethyl acetate extract has a strong ferric-ion reducing ability, which is related to the polarity of the solvent. The greater the polarity, the stronger the reducing ability, however, the water extract had the weakest reducing ability, which might be due to the extraction of water-soluble chemical components with water as the extraction solvent, resulting in the reduced antioxidant activity of the extract.

Flavonoid characterization of fig leaves by HPLC-DAD-ESI-MS

Flavonoids from fig waste leaves were characterized by HPLC-DAD-ESI-MS, as is shown in figure 4, 11 negative peaks of ethyl acetate extract in fig leaves were observed at 517 nm. By scanning in negative ion mode, 11 compounds were analyzed by mass spectrometry. The larger the area of the negative peak, the stronger the antioxidant activity of the compound. The chemical structures of 11 compounds are elucidated in figure 5. Among them, 1, 6 and 7 are the main substances with high antioxidant activity. It is speculated that they are 3-O-(rhamnopyranosyl-glucopyranosyl)-7-O-(glucopyranosyl)-quercetin, isoschaftoside and rutin, respectively.

Under the condition of negative ions, the total ion flow diagram of flavonoids in fig leaves is shown in figure 6. Through the analysis of the information in the first-level mass spectrometry, it can be preliminarily inferred that the molecular weight of each compound is shown in Table 1. By comparing the fragmentation information of the target compounds in the secondary mass spectrometry and searching the computer standard mass spectrometry database, combining with the references, the experimental results were comprehensively analyzed [34].

Radical scavenging capacity of fig flavonoids by online HPLC-DPPH

The isolated compound reacted with DPPH in post-column, and the antioxidants in fig leaves were screened. 11 components of fig leaves extract have negative peak can be observed in DPPH free radical detection spectrum at wavelength 517 nm (figure 4). Three main peaks (1, 6 and 7) were clearly observed as the main contributors of antioxidants. The results showed that there were abundant antioxidant substances scavenging DPPH free radical in fig leaves. When extracted with ethyl acetate, the negative peak had a better front shape, a stable baseline and a large signal noise ratio (SNR).

Identification of flavonoid compounds

The active components in ethyl acetate from fig leaves were identified according to other characteristics of fragments in the mass spectrometry. 11 flavonoids in fig leaves were identified and their chemical structures were determined. Table 1 presented the retention time, MS fragmentation and molecular formula of compounds detected by HPLC-QTOF-MS/MS analysis. The first-level and secondary mass spectrometry of 11 flavonoids were shown in figure 7.

Compound 1: from the first-level mass spectrometry of compound 1, it can be found that there is an excimer ion peak at m/z 771.2027 in the negative ion mode. The main fragment ions were obtained by secondary scanning of the parent ion m/z 771.2027, which were 609.1530, 462.0838 and 301.0357, respectively. According to the mass spectrometry data in Table 1 and related literature [35], it was inferred that the compound is 3-O-(rhamnopyranosyl-glucopyranosyl)-7-O-(glucopyranosyl)-quercetin.

Compound 2: the m/z 365.0881 of the negative ion mode scan was a $[M-H]^-$ peak. The main fragments were 203.0352, 159.0454, 130.0422 by secondary mass spectrometry scanning of m/z 365.0881 parent ion. According to the fragmentation information combined with the literature [36], the compound was identified as 2-carboxyl-1, 4-naphthohydroquinone-4-O-glucopyranoside according to the mass spectrometry data in Table 1.

Compound 3: the m/z 579.1366 scanning in negative ion mode was $[M-H]^-$ peak. The main fragments were 519.1194, 489.1083, 429.0856, 399.0748, 369.0635 and 339.0521 by the secondary mass spectrometry scanning of the parent ion m/z 579.1419. It was found to be luteolin 6-C-glucopyranoside, 8-C-arabinopyranoside according to the mass spectrometry data in Table 1 and related literature [37].

Compound 4: in the first-level mass spectrometry of the compound, the m/z 563.1412 scanning in the negative ion mode of the compound was $[M-H]^-$ peak. The main secondary mass fragments were m/z 443.1001, 473.1115, 353.0670 and 383.0795 by the secondary mass spectrometry scanning of the parent ion m/z 563.1414. According to the mass spectrometric data in Table 1 and the information combined with the previous literature [38], the compound was presumed to be schaftoside.

Compound 5: the m/z 447.0934 scanning in negative ion mode was $[M-H]^-$ peak. Through the secondary mass spectrometry scanning of the parent ion m/z 447.0937, the main secondary mass spectrometry fragments can be obtained as follows: 369.0615, 357.0622, 327.0511, 297.0397, 285.0396 and 133.0280. According to the mass spectrometric data in Table 1 and related literature [39], it was speculated that compound 5 was isoorientin.

Compound 6: the m/z 563.1412 scanning in negative ion mode of the compound was $[M-H]^-$ peak. The main fragments were m/z 443.1001, 473.1115, 353.0670 and 383.0795 by the secondary mass spectrometry scanning of the parent ion m/z 563.1414. According to the mass spectrometric data in Table 1 and related literature [40], it was speculated that this compound was isoschaftoside.

Compound 7: the m/z 609.1470 of negative ion mode scanning is $[M-H]^-$ peak. The main secondary mass spectrometry fragments were 301.0362, 151.0031, 257.0450 and 273.0477 by the secondary mass spectrometry scan of the parent ion m/z 609.1470. According to the mass spectrum data in Table 1 and related literature [41], the compound was presumed to be rutin.

Compound 8: the negative ion mode scan m/z 577.1577 shows the $[M-H]^-$ peak. By the secondary mass spectrometry scanning of the parent ion m/z 577.1577, the main secondary mass spectrometry fragments were obtained as m/z 457.1164 and 293.0454. The compound was presumed to be 2"-O-rhamnosylvitexin according to the mass spectrum data in Table 1 and related literature [42].

Compound 9: scanning m/z 431.0989 in the negative ion mode of the compound showed $[M-H]^-$ peak. The main fragments of m/z 431.0981 were 283.0621, 311.0564 and 341.0673 by secondary mass spectrometry. According to the mass spectrum data in Table 1 and related literature [43], it could be speculated that the compound was isovitexin.

Compound 10: scanning in negative ion mode at m/z 463.0890 showed $[M-H]^-$ peak. m/z 463.0890 was scanned by secondary mass spectrometry, and the main fragment ions were m/z 301.0386 and 151.0033. According to the mass spectrum data in Table 1 and earlier studies [44], the compound was presumed to be isoquercetin.

Compound 11: scanning m/z 593.1524 in the negative ion mode of the compound showed $[M-H]^-$ peak. m/z 593.1576 was scanned by secondary mass spectrometry, and the main fragment ion was m/z 285.0403. The compound was presumed to be kaempferol-3-O-rutinoside according to the mass spectrum data in Table 1 and unified with the literature [45].

Conclusions

In this study, online HPLC-QTOF-MS/MS-DPPH method was used for the first time to screen and identify 11 antioxidant active components in fig waste leaves, including 3-O-(rhamnopyranosyl-glucopyranosyl)-7-O-(glucopyranosyl)-quercetin (1), 2-carboxyl-1, 4-naphthohydroquinone-4-O-glucopyranoside (2), luteolin 6-C-glucopyranoside, 8-C-arabinopyranoside (3), schaftoside (4), isoorientin (5), isoschaftoside (6), rutin (7), 2"-O-rhamnosylvitexin (8), isovitexin (9), isoquercetin (10), kaempferol-3-O-rutinoside (11), respectively. The antioxidant capacities of different extracts were based on DPPH and ABTS free radical scavenging rate and FRAP reduction ability. The ethyl acetate extract had the strongest antioxidant capacity. Furthermore, through online HPLC-DPPH analysis, compounds (1), (6) and (7) were considered to be significant antioxidant activity. Therefore, online HPLC-QTOF-MS/MS-DPPH was an effective and rapid analysis method for determining the antioxidant capacity of fig waste leaves, which provided the data support for development and utilization of fig resources.

Declarations

Acknowledgements The author thanks Rongcheng healthy group co., ltd for supplying the fig waste leaves.

Funding This work was financially supported by the Fundamental Research Fund for Central Universities (2572019CZ01) and Heilongjiang Touyan Innovation Team Program (Tree Genetics and Breeding Innovation Team).

Declarations

Conflicts of Interest The authors have declared no conflict of interest.

Availability of data and material Not applicable

Code availability Not applicable

Authors' Contributions Chunjian Zhao and Yujie Fu conceived the underlying ideas of the study, Chunying Li, Meiting Yu and Shen Li contributed to the practical aspects of the research work, Xue Yang, Bin Qiao and Sen Shi analyzed the data, and Chunying Li, Meiting Yu and Shen Li wrote the manuscript.

References

1. Jules, J.: The Fig: Horticultural Reviews. John Wiley & Sons Inc, New Jersey (2010)
2. Zohary, D., Spiegel-Roy, P.: Beginnings of Fruit Growing in the Old World. *Science*. **187**, 319–327 (1975)
3. Afsah-Hejri, L., Toudeshki, A., Homayouni, T., Mehrazi, S., Ghomlami Pareh, A., Gordon, P., Ehsani, R.: Potential of ozonated-air (OA) application to reduce the weight and volume loss in fresh figs (*Ficus*

- carica L.). Postharvest Biol Technol. **180**, 111631 (2021)
4. Abdel-Rahman, R., Ghoneimy, E., Abdel-Wahab, A., Eldeeb, N., Salem, M., Salama, E., Ahmed, T.: The therapeutic effects of *Ficus carica* extract as antioxidant and anticancer agent. *S. Afr. J. Bot.* **141**, 273–277 (2021)
 5. Muhammad, N., Alam, Z.: Impact of maturity on phenolic composition and antioxidant activity of medicinally important leaves of *Ficus carica* L. *Physiol. Mol. Biol. Plants.* **24**, 1–7 (2018)
 6. Li, Z., Yang, Y., Liu, M., Zhang, C., Cui, Q.: A comprehensive review on phytochemistry, bioactivities, toxicity studies, and clinical studies on *Ficus carica* Linn. leaves. *Biomed. Pharmacother.* **137**, 111393 (2021)
 7. Mahmoudi, S., Khali, M., Benkhaled, A., Benamirouche, K., Baiti, I.: Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian *Ficus carica* L. varieties. *Asian Pac. J. Trop. Biomed.* **6**, 239–245 (2016)
 8. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J.: Free radicals and antioxidants in normal physiological functions and human disease - ScienceDirect. *Int. J. Biochem. Cell Biol.* **39**, 44–84 (2007)
 9. Alcántara, C., Ugi, T., Abdelkebir, R., García-Pérez, J., Jambrak, A.R., Lorenzo, J.M., Collado, M.C., Granato, D., Barba, F.J.: Effects of ultrasound-assisted extraction and solvent on the phenolic profile, bacterial growth, and anti-Inflammatory/antioxidant activities of mediterranean olive and Fig Leaves extracts. *Molecules.* **25**, 1718 (2020)
 10. Sánchez-Valdeolivar, C., Alvarez-Fitz, P., Zacapala-Gómez, A., Acevedo-Quiroz, M., Mendoza-Catalán, M.: Phytochemical profile and antiproliferative effect of *Ficus crocata* extracts on triple-negative breast cancer cells. *BMC Complementary Med. Ther.* **20**, 191 (2020)
 11. Canal, J.R., Torres, M., Romero, A., Pérez, C.: A chloroform extract obtained from a decoction of *Ficus carica* leaves improves the cholesterolaemic status of rats with streptozotocin-induced diabetes. *Acta Physio. Hung.* **87**, 71–76 (2000)
 12. Konyalioglu, S., Saglam, H., Kivcak, B.: α -tocopherol, flavonoid, and phenol contents and antioxidant activity of *Ficus carica*. leaves. *Pharm. Biol.* **43**, 683–686 (2008)
 13. Ali, B., Mujeeb, M., Aeri, V., Mir, S.R., Faiyazuddin, M., Shakeel, F.: Anti-inflammatory and antioxidant activity of *Ficus carica* Linn. leaves. *Nat. Prod. Res.* **26**, 460–465 (2012)
 14. Park, S., Han, J., Im, K., Whang, W.K., Min, H.: Antioxidative and anti-inflammatory activities of an ethanol extract from fig (*Ficus carica*) branches. *Food Sci. Biotechnol.* **22**, 1071–1075 (2013)
 15. Canal, C.Pérez, Campillo, J.R., Romero, J.E., Torres, A.M.D.: Hypotriglyceridaemic activity of *Ficus carica* leaves in experimental hypertriglyceridaemic rats. *Phytother. Res.* **13**, 188–191 (1999)
 16. Pérez, C., Domínguez, E., Ramiro, J.M., Romero, A., Campillo, J.E., Torres, M.: A study on the glycaemic balance in streptozotocin diabetic rats treated with an aqueous extract of *Ficus carica* (fig tree) leaves. *Phytother. Res.* **10**, 82–83 (1996)
 17. Serracarla, A., Hawkins, F., Pérez, C., Domnguez, E., Campillo, J.E., Torres, M.D.: Hypoglycemic action of an oral fig-leaf decoction in type-I diabetic patients 1. *Diabetes Res. Clin. Pract.* **39**, 19–22 (1998)

18. Arvaniti, O.S., Samaras, Y., Gatidou, G., Thomaidis, N.S., Stasinakis, A.S.: Review on fresh and dried figs: Chemical analysis and occurrence of phytochemical compounds, antioxidant capacity and health effects. *Food Res. Int.* **119**, 244–267 (2019)
19. Garcia, C., Blesso, C.N.: Antioxidant properties of anthocyanins and their mechanism of action in atherosclerosis. *Free Radical Biol. Med.* **172**, 152–166 (2021)
20. Lijuan, Y., Na, Z., Chenbiao, W., Chunhong, W.: Highly selective separation and purification of anthocyanins from bilberry based on a macroporous polymeric adsorbent. *J. Agric. Food Chem.* **63**, 3543–3550 (2015)
21. Bu, Y., Lee, K., Jung, H.S., Moon, S.K., Pharmacology, D., Medicine, C., University, K.H., Anatomy, D.O., Medicine, I., Disease, C.N.: Therapeutic effects of traditional herbal medicine on cerebral ischemia: A perspective of vascular protection. *Chi. J. Integr. Med.* **19**, 804–814 (2013)
22. Maron, D.J.: Flavonoids for reduction of atherosclerotic risk. *Curr. Atheroscler. Rep.* **6**, 73–78 (2004)
23. Li, Y.M., Fan, Y., Liu, Y., Hao, M.F., Li, X., Liu, L., Zi, H., Li, J.: Effect of compatibility of astragalus flavonoids and kudzu flavonoids on glucose and lipid metabolism in liver tissue. *Chin. J. Exp. Tradit. Med. Formulae.* **21**, 109–112 (2015)
24. Vazquez-Olivo, G., Lopez-Martinez, L.X., Contreras-Angulo, L., Basilio Heredia, J.: Antioxidant capacity of lignin and phenolic compounds from corn stover. *Waste Biomass Valorization* **10**, 95–102 (2019)
25. Kedare, S.B., Singh, R.P.: Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* **48**, 412–422 (2011)
26. Okur, I., Baltacioglu, C., Agcam, E., Baltacioglu, H., Alpas, H.: Evaluation of the effect of different extraction techniques on sour cherry pomace phenolic content and antioxidant activity and determination of phenolic compounds by FTIR and HPLC. *Waste biomass valorization* **10**, 3545–3555 (2019)
27. Brito, E., Araújo, M.C.P.D., Lin, L.Z., Harnly, J.: Determination of the flavonoid components of cashew apple (*Anacardium occidentale*) by LC-DAD-ESI/MS. *Food Chem.* **105**, 1112–1118 (2007)
28. Nuengchamnong, N., Ingkaninan, K.: On-line HPLC-MS-DPPH assay for the analysis of phenolic antioxidant compounds in fruit wine: *Antidesma thwaitesianum* Muell. *Food Chem.* **118**, 147–152 (2010)
29. Shi, P., Du, W., Wang, Y., Teng, X., Chen, X., Ye, L.: Total phenolic, flavonoid content, and antioxidant activity of bulbs, leaves, and flowers made from *Eleutherine bulbosa* (Mill.) Urb. *Food Sci. Nutr.* **7**, 148–154 (2018)
30. Zhang, L., Ding, X.P., Qi, J., Yu, B.Y.: Determination of antioxidant activity of tea by HPLC-DPPH. *J. China Pharm. Univ.* **43**, 236–240 (2012)
31. Han, Z., Zhang, J., Cai, S., Chen, X., Quan, X., Zhang, G.: Association mapping for total polyphenol content, total flavonoid content and antioxidant activity in barley. *BMC Genomics.* **19**, 81 (2018)
32. Neupane, P., Lamichhane, J.: Estimation of total phenolic content, total flavonoid content and antioxidant capacities of five medicinal plants from Nepal. *Vegetos.* **33**, 360–366 (2020)

33. Jin, L., Li, X.B., Tian, D.Q., Fang, X.P., Yu, Y.M., Zhu, H.Q., Ge, Y.Y., Ma, G.Y., Wang, W.Y., Xiao, W.F., Li, M.: Antioxidant properties and color parameters of herbal teas in China. *Ind. Crops Prod.* **87**, 198–209 (2016)
34. Belguith-Hadriche, O., Ammar, S., Contreras, M.d.M., Fetoui, H., Segura-Carretero, A., El Feki, A., Bouaziz, M.: HPLC-DAD-QTOF-MS profiling of phenolics from leaf extracts of two Tunisian fig cultivars: Potential as a functional food. *Biomed. Pharmacother.* **89**, 185–193 (2017)
35. Adjé, F., Lozano, Y.F., Gernevé, C., Lozano, P.R., Meudec, E., Adima, A.A., Gaydou, E.M.: Phenolic acid and flavonol water extracts of *Delonix regia* red flowers. *Ind. Crops Prod.* **37**, 303–310 (2012)
36. Zhou, J., Xie, G., Yan, X.: *Encyclopedia of traditional Chinese medicines - molecular structures, pharmacological activities, natural sources and applications.* Springer-Verlag, Berlin (2011)
37. Qasim, M., Muhammad, H., Ikram, A.: Antioxidant and antimicrobial activities of *Ixora coccinea* root and quantification of phenolic compounds using HPLC. *S. Afr. J. Bot.* **135**, 71–79 (2020)
38. Zhang, L.H., Li, Q.G.: Analysis of electrospray ionization cracking law of schaftoside. *Chin. Med. Sci.* **6**, 52–54 + 137 (2016)
39. Deng, S.S., Liu, H.X., Ma, L.H., Wang, T.T., Wang, Y.D., Huang, X.P.: Qualitative analysis and HPLC determination of flavonoids in Leaves of *Cymbiditis cerevissima* L. by UPLC-MS/MS. *China Med. Her.* **15**, 80–88 (2018)
40. Fernando, W., Attanayake, A., Perera, H., Sivakanesan, R., Fujimoto, Y.: Isolation, identification and characterization of pancreatic lipase inhibitors from *Trigonella foenum-graecum* seeds. *S. Afr. J. Bot.* **121**, 51–57 (2019)
41. Yang, J., Qian, D., Jiang, S., Shang, E.X., Guo, J., Duan, J.A.: Identification of rutin deglycosylated metabolites produced by human intestinal bacteria using UPLC-Q-TOF/MS. *J. Chromatogr. B* **898**, 95–100 (2012)
42. Joea, B., Ago, A., Mo, A., Maaa, C., Mg, A.: Pharmacological evaluation of hydro-ethanol and hot water leaf extracts of *Bauhinia galpinii* (Fabaceae): A South African ethnomedicinal plant. *S. Afr. J. Bot.* **128**, 28–34 (2020)
43. Li, Y.X., Zhang, Y.Q., Yang, T., Li, H., Guo, J., Zhao, Q.Q., Xie, J.B.: Pharmacokinetics and tissue distribution study of Isovitexin in rats by HPLC-MS/MS. *J. Chromatogr. B* **991**, 13–20 (2015)
44. Yan, Z., Zhang, H., Dzah, C.S., Zhang, J., Duan, Y.: Subcritical water extraction, identification, antioxidant and antiproliferative activity of polyphenols from lotus seedpod. *Sep. Purif. Technol.* 116217 (2020)
45. Wang, Y., Tang, C., Zhang, H.: Hepatoprotective effects of kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside from *Carthamus tinctorius* L. on CCl₄-induced oxidative liver injury in mice. *J. Food Drug Anal.* **23**, 310–317 (2015)

Table

Tab.1 Flavonoids identified in the fig leaves extracts using HPLC-QTOF-MS/MS.

Peak no.	Retention time (min)	First-level mass spectrometry	Secondary mass spectrometry	Molecular weight	Molecular formula	Identification
1	10.349	771.2027	609.1530, 462.0838, 301.0357	772.2027	C ₃₃ H ₄₀ O ₂₁	3-O-(rhamnopyranosyl-glucopyranosyl)-7-O-(glucopyranosyl)-quercetin
2	11.706	365.0881	203.0352, 159.0454, 130.0422	366.0881	C ₁₇ H ₁₈ O ₉	2-carboxyl-1, 4-naphthohydroquinone-4-O-glucopyranoside
3	11.835	579.1366	519.1194, 489.1083, 429.0856, 369.0635	580.1366	C ₂₆ H ₂₈ O ₁₅	luteolin 6-C-glucopyranoside, 8-C-arabinopyranoside
4	12.706	563.1414	473.1115, 443.1001, 353.0670	564.1412	C ₂₆ H ₂₈ O ₁₄	schaftoside
5	13.007	447.0934	369.0615, 357.0622, 327.0511, 297.0397, 285.0396, 133.0280	448.0934	C ₂₁ H ₂₀ O ₁₁	isoorientin
6	13.164	563.1412	443.1001, 353.0670, 473.1115, 383.0795	564.1412	C ₂₆ H ₂₈ O ₁₄	isoschaftoside
7	14.018	609.1470	301.0362, 151.0031, 257.0450, 273.0477	610.1470	C ₂₇ H ₃₀ O ₁₆	rutin
8	14.189	577.1577	457.1164, 293.0454	578.1577	C ₂₇ H ₃₀ O ₁₄	2"-O-rhamnosylvitexin
9	14.275	432.1056	341.0673, 311.0564, 283.0612	432.0989	C ₂₁ H ₂₀ O ₁₀	isovitexin
10	14.447	463.0890	301.0357, 151.0033	464.0890	C ₂₁ H ₂₀ O ₁₂	isoquercetin
11	15.402	593.1524	285.0403	594.1524	C ₂₇ H ₃₀ O ₁₅	kaempferol-3-O-rutinoside

Figures

Figure 1

The flow chart of online HPLC-DPPH screening system.

Figure 2

Effect of different extraction solvent on the yield of total polyphenol and flavonoids. (a. polyphenol; b. flavonoids).

Figure 3

Effect of different extraction solvent on antioxidant capacity. (a. scavenging capacity on DPPH radical; b. scavenging capacity on ABTS radical; c. ferric-ion reducing capacity).

Figure 4

Antioxidant components of ethyl acetate extract from fig leaves by online HPLC-DPPH. (1. 3-O-(rhamnopyranosyl-glucopyranosyl)-7-O-(glucopyranosyl)-quercetin; 2. 2-carboxyl-1, 4-naphthohydroquinone-4-O-glucopyranoside; 3. luteolin 6-C-glucopyranoside, 8-C-arabinopyranoside; 4. schaftoside; 5. isoorientin; 6. isoschaftoside; 7. rutin; 8. 2"-O-rhamnosylvitexin; 9. isovitexin; 10. isoquercetin; 11. kaempferol-3-O-rutinoside).

Figure 5

Chemical structures of 11 compounds in fig leaves. (1. 3-O-(rhamnopyranosyl-glucopyranosyl)-7-O-(glucopyranosyl)-quercetin; 2. 2-carboxyl-1, 4-naphthohydroquinone-4-O-glucopyranoside; 3. luteolin 6-C-glucopyranoside, 8-C-arabinopyranoside; 4. schaftoside; 5. isoorientin; 6. isoschaftoside; 7. rutin; 8. 2"-O-rhamnosylvitexin; 9. isovitexin; 10. isoquercetin; 11. kaempferol-3-O-rutinoside).

Figure 6

Total ion flow chromatogram of sample extract. Peak 1-11 are identified in Table 1.

Figure 7

The mass spectrometry of flavonoids compounds. (a. 3-O-(rhamnopyranosyl-glucopyranosyl)-7-O-(glucopyranosyl)-quercetin; b. 2-carboxyl-1, 4-naphthohydroquinone-4-O-glucopyranoside; c. luteolin 6-C-glucopyranoside, 8-C-arabinopyranoside; d. schaftoside; e. isoorientin; f. isoschaftoside; g. rutin; h. 2''-O-rhamnosylvitexin; i. isovitexin; j. isoquercetin; k. kaempferol-3-O-rutinoside).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicAbstract.pdf](#)