In Vitro Evaluation of the Chemical Composition and Various Biological Activities of Ficus carica Leaf Extracts

Ficus carica Yaprak Ekstrelerinin Kimyasal Bileşiminin ve Çeşitli Biyolojik Aktivitelerinin İn Vitro Değerlendirilmesi

Mustafa ERGÜL*, Merve ERGÜL, Nuraniye ERUYGUR, Mehmet ATAŞ, Esra UÇAR

1Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Biochemistry, Sivas, Turkey
2Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmacology, Sivas, Turkey
3Selçuk University, Faculty of Pharmacy, Department of Pharmacognosy, Konya, Turkey
4Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Microbiology, Sivas, Turkey
5Sivas Cumhuriyet University, Sivas Vocational School, Department of Medicinal and Aromatic Plants, Sivas, Turkey

ABSTRACT

Objectives: The present study aimed to investigate the inhibitory activities of enzymes related to diabetes mellitus and Alzheimer’s disease of the methanol and water extracts of Ficus carica leaf extracts. The bioactive compounds and anticancer, antioxidant, and antimicrobial effects of the extracts were also investigated.

Materials and Methods: The bioactive compounds in the extracts were determined by gas chromatography-mass spectrometry. The antioxidant activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2’-azino-bis(3-ethylbenzothiazoline-6 sulphonic acid) (ABTS) radical scavenging, total phenol and flavonoid content, ferric reducing power, and iron chelating method. The anticancer, anticholinesterase, and antimicrobial effects were investigated using the XTT assay, Ellman method, and microdilution, respectively.

Results: Our results showed that between the water and methanol extracts there was a difference in terms of chemical composition. The antioxidant results suggested that both extracts have strong antioxidant activity. Similarly, both extracts showed strong α-glucosidase and α-amylase inhibition activity, while the water extract had higher inhibition activity than the methanol extract against acetylcholinesterase and butyrylcholinesterase. The methanol extract of F. carica exhibited significant anticancer activity on MDA-MB-231 cells and showed moderate antimicrobial activities against Escherichia coli and Staphylococcus aureus.

Conclusion: Our results suggest that F. carica leaves could be a valuable source for developing a promising therapeutic agent in cancer, diabetes, and Alzheimer’s disease.

Key words: Ficus carica, Alzheimer’s disease, diabetes, antioxidant activity, anticancer and antimicrobial activities

ÖZ

Amaç: Bu çalışmada, Ficus carica yaprak ekstrelerinin inhibitory etkileri üzerinde araştırılmıştır. Ayrıca, ekstrelerin antioxidant aktiviteleri, antikanser, antidiabetik ve antimikrobiyel aktiviteleri de incelenmiştir.

Gereç ve Yöntemler: Ekstrelerin antioxidant etkileri ve kimyasal bileşimleri, gas chromatography-mass spectrometry (GC-MS) yöntemi ile belirlenmiştir. Antioxidant ve antidiabetik aktiviteleri 1,1-diphenyl-2-picrylhydrazyl (DPPH) ve 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) yöntemleri ile değerlendirilmiştir. Antikanser etkisi XTT ve Ellman yöntemleriyle, antimikrobiyel etkisi ise microdilution yöntemi ile değerlendirilmiştir.

Bulgular: Çalışmamıza göre, Ficus carica yaprak ekstrelerinin antioxidant etkileri ve kimyasal bileşimleri, antidiabetik ve antikanser etkileri açısından iyi bir değer göstermektedir. Ekstrelerin antioxidant etkisi ve kimyasal bileşimleri, antidiabetik etkileri ve antioxidant etkileri açısından iyi bir değer göstermektedir. Ekstrelerin antimikrobiyel etkisi ise, microdilution yöntemi ile değerlendirildiğiinde iyi bir değeri göstermektedir.

Sonuç: Bulgularımız, Ficus carica yaprak ekstrelerinin antioxidant, antidiabetik ve antikanser etkileri açısından iyi bir değer gösterdiğini göstermektedir. Bu bulgular, Ficus carica yaprak ekstrelerinin, diyetetik ve farmakolojik öneme sahip olduğunu göstermektedir.

Anahtar kelimeler: Ficus carica, antioxidant, antidiabetik, antikanser, antimikrobiyel aktiviteleri

*Correspondence: E-mail: m.ergul@yahoo.com.tr, Phone: +90 555 691 46 67 ORCID: orcid.org/0000-0003-4303-2996
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INTRODUCTION

*Ficus carica* L. belongs to the family Moraceae and is a native of southwest Asia. It is cultivated worldwide and has been traditionally used in indigenous systems of medicine, such as Ayurveda and homeopathy, for cardiovascular and hypertensive diseases. Its fruit possesses several vitamins, minerals, carbohydrates, and phenolic compounds, for instance, phenolic acids, flavonols, and flavones, which play a significant role in its therapeutic efficiency. Many reports also stated that the polyphenolic ingredient of the fruit has anti-inflammatory, antioxidant, antimicrobial, and anticancer effects. In recent years, due to increasing cancer cases and similar health problems, the demand for products with antioxidant properties has been increasing day by day. In this context, plants that have antioxidant and anticancer properties have attracted wide attention. It is well known that antioxidants have significant inhibitory effects on various free radical species and also neutralize nonradical species such as hydrogen peroxide. Additionally, they can prevent the production of many reactive oxygen species in various diseases such as cancer and diabetes.

Diabetes mellitus is a chronic metabolic disease that causes elevation of blood sugar due to insufficient insulin secretion or insulin resistance. α-Glucosidase and α-amylase inhibitors are used in some cases to control the level of postprandial blood glucose in the treatment of diabetes mellitus. These two enzymes are involved in the conversion of food polysaccharides into monosaccharides. However, synthetic hypoglycemic agents are involved in the conversion of food polysaccharides into monosaccharides. Accordingly, the current treatment strategy for AD is directed to the inhibition of AChE and BChE. There are some AChE inhibitors such as galantamine, phystostigmine, and tacrine approved for the treatment of AD. However, these drugs have side effects, including hepatotoxicity, limiting the use of these drugs in clinical practice. Hence, researchers are looking for new treatments to control the disease and improve the quality of life for people with AD from natural resources.

Alzheimer’s disease (AD) is the most common form of dementia, characterized by memory loss and other cognitive disabilities. Down-regulation of acetylcholine is associated with the development of AD. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are responsible for the hydrolytic metabolism of the neurotransmitter acetylcholine (ACh) into choline and acetate in the brain. Based on the cholinergic hypothesis, a defect in the cholinergic system is involved in the development of AD. Therefore, the current treatment strategy for AD is directed to the inhibition of AChE and BChE. There are some AChE inhibitors such as galantamine, phystostigmine, and tacrine approved for the treatment of AD. However, these drugs have side effects, including hepatotoxicity, limiting the use of these drugs in clinical practice. Hence, researchers are looking for new treatments to control the disease and improve the quality of life for people with AD from natural resources.

Cancer is one of the most significant health issues worldwide and the second leading cause of death globally after cardiovascular diseases. Conventional treatments used in the clinic such as chemotherapy, surgery, and radiotherapy have several serious side effects and can cause damage to noncancerous tissues. Moreover, due to increasing drug resistance especially in cancer treatment, plants have become increasingly important in the search for new chemotherapeutic agents. In the clinic, there are many antitumor drugs derived from plants such as vincristine, vinblastine (*Catharanthus* sp.), paclitaxel (*Taxus* sp.), and epipodophyllotoxins (*Podophyllum* sp.). Furthermore, research continues at a great pace for the discovery of new drugs with more effective and less side effect profiles. *F. carica* is one of the medicinally important plants with therapeutic potential. Many researchers have reported the antimicrobial effects of *F. carica* leaf extracts against oral bacteria, nosocomial infectious agents, food poisoning bacteria, fungi, and viruses. Moreover, the fruit, root, and leaves of *F. carica* are utilized medicinally for treating various diseases as a respiratory, gastrointestinal, anti-inflammatory, and antispasmodic remedy.

To the best of our knowledge, the fruit and different parts of this plant have been mostly studied, and the number of study on the leaves is limited. Thus, this study was carried out to evaluate differences between water and methanol extracts for antioxidant, antimicrobial, enzyme inhibition activity (AChE, BChE, α-glucosidase, and α-amylase), and anticancer properties *in vitro*. It was also aimed to analyze the content of extracts by gas chromatography-mass spectroscopy (GC-MS).

EXPERIMENTAL

This study was conducted in the laboratories of the Faculty of Pharmacy, Sivas Cumhuriyet University, Sivas, in 2018. The plant materials were collected in July 2017 from the wild flora of Sakklikent/Fethiye. The experiments were performed in a completely randomized design with three replications.

Preparation of extracts

The plant leaves were milled with a grinder and then dried in the shade and the dry leaves were ground in a blender (Blue house). Ten grams of the leaf was soaked in 50 mL of methanol (Sigma) and water for 24 h with intermittent shaking. At the end of the extraction, it was filtered through No. 1 Whatman filter paper. The filtrate was concentrated to dryness under reduced pressure in a rotary evaporator at 40°C and this was repeated three times. The obtained extracts were analyzed using GC-MS.

In vitro antioxidant activity

The antioxidant activity of the methanol and water extracts of *F. carica* leaves was tested using different methods, namely DPPH, ABTS radical scavenging activity, total phenol/flavonoid content, ferric reducing power, and iron chelating method.

DPPH radical scavenging activity

The free radical scavenging activity by methanol extracts was determined according to the method reported by Miser Salihoglu et al. First 150 µL of the extract was mixed with 50 µL of 1.0×10⁻³ M freshly prepared DPPH• methanol solution in 96-well plates. Methanol was used as the control of the experiment. After 30 min of incubation at 25°C, the reduction of the DPPH• was measured reading the absorbance at 517 nm with a microplate reader (Epoch, USA). Butylated hydroxytoluen (BHT) was used for the positive controls and the percentage inhibition was calculated with the following equation:
% Inhibition=[Absorbance of control−Absorbance of test sample/Absorbance of control]×100

**ABTS radical scavenging activity**

For determining the ABTS radical scavenging activity of the extracts, the method described by Re et al. was followed with slight modification. The stock solution of ABTS was made by reacting 7 mM ABTS solution with 2.4 mM potassium persulfate solution in equal volume for 16 h. The working solution was then prepared by diluting the stock ABTS solution with methanol to give an absorbance of 0.7±0.02 units at 734 nm using a microplate reader (Epoch, USA). In each experiment, the ABTS solution was prepared freshly. Fifty microliters of the extract in DMSO was mixed with 100 µL of the ABTS•+ solution was prepared freshly. Fifty microliters of working solution were mixed together in 96-well plates and incubated for 30 min at 25°C. The measurement of absorbance was performed at 593 nm. A standard calibration curve was prepared using different concentrations of FeSO4 solution. The results were expressed as FRAP values.

**Ferric reducing antioxidant power (FRAP) assay**
The FRAP reagent is used as a reducing agent in redox colorimetric reactions of antioxidants. The FRAP assay was conducted according to the previously reported method with a slight modification. The stock solution of each extract was prepared in DMSO. The working solution of FRAP reagent was prepared by mixing 0.3 M pH 3.6 acetate buffer and a solution of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 0.04 M HCl and 0.02 M FeCl3 solution in the ratio of 10:1:1 at the time of use. All solutions were prepared fresh on the day of the experiment. Thirty microliters of the sample solution and 270 µL of FRAP working solution were mixed together in 96-well plates and warmed at 37°C for 4 min. All determinations were performed in triplicate. The absorbance was measured at 593 nm. A standard calibration curve was prepared using different concentrations of FeSO4 solution. The results were expressed as FRAP values.

**Acetylcholinesterase/butyrylcholinesterase inhibition assay**
The assay was carried out according to the Ellman method as follows. The mixture consisting of 20 µL of test sample/reference standard of various concentrations, 140 µL of 0.1 mM phosphate buffer (pH 6.8), 10 µL of 3 mM 5,5'-dithio-bis-nitrobenzoic acid (DTNB), and 20 µL of enzyme (0.22 U/mL for acetylcholinesterase/0.1 U/mL for butyrylcholinesterase) prepared in phosphate buffer was incubated for 5 min at 25°C. Following preincubation, 10 µL of the substrate (0.71 mM acetylthiocholine iodide/0.2 mM butyrylthiocholine chloride in phosphate buffer) was added to start the reaction, followed by further incubation for 10 min. The developed yellow color was measured at 412 nm (Epoch, USA). Galantamine was used as the positive control.

**Alpha-glucosidase inhibition activity**
The α-glucosidase inhibition method was reported by Kumar et al. Ascarbose was used as a positive control, while phosphate buffer was used as a negative control in place of the sample. Each concentration was carried out in triplicate. Twenty-five microliters of sample solution diluted with buffer was mixed with 25 µL of α-glucosidase (0.5 U/mL) and incubated for approximately 10 min at 25°C. Then 25 µL of 0.5 mM 4-nitrophenyl-β-D-glucuronide (pNPG) was added to each well as substrate and incubated for 30 min at 37°C. After the incubation period, 100 µL of 0.2 M sodium carbonate was added to terminate the reaction and the absorbance was read at 405 nm.

**Alpha-amylase inhibition activity**
The α-amylase inhibition method was reported by Kumar et al. Acarbose was used as a positive control, while phosphate buffer (0.02 M PBS, pH 6.9) was used as a negative control in place of the sample. Each sample was tested in triplicate with different concentrations. The reaction mixture containing 50 µL of sample solution diluted with buffer and 25 µL of α-amylase from porcine pancreases (0.5 mg/mL) was incubated for approximately 10 min at 25°C. Then 50 µL of freshly prepared 0.5% starch solution (w/v) was added to each well as substrate and incubated for 10 min at 25°C. After the incubation period,
100 µL of 1% 3,5-dinitrosalicylic acid (DNS) was added as the color reagent, followed by heating in a water bath for 10 min. The absorbance was read at 540 nm.

**Antimicrobial activity**

**Microdilution broth method**

The microdilution broth method with slight modification was used to determine the minimum inhibitory concentration (MIC) of the water and methanol extracts of *F. carica* against the microorganism. In the present study, *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), and *Candida albicans* (ATCC 10231) strains were used. The extracts were dissolved in 50% dimethyl sulfoxide (DMSO) and the final concentrations of the extracts were 50 µg/mL. Mueller Hinton Broth (Accumix® AM1072) and Sabouraud Dextrose Broth (Himedia ME033) were used for dilution bacteria and *C. albicans* cultures, respectively. In the first row of the plate, 90 µL of broth was added to the wells and 50 µL of broth was added to all other wells. The 11th wells were used as the reproductive controls and 100 µL of broth was added. In the first line of the microtiter plate, 10 µL of extract was added and serial two-fold dilutions were prepared from the diluted extracts to give concentrations ranging from 2.5 to 0.004 mg/mL. The bacteria and fungi suspensions (50 µL) were added to prepared samples. The final inoculum size was 5×10³ CFU/mL in the bacteria wells and 0.5–2.5×10³ CFU/mL in the *C. albicans* wells (CLSI, 2002, CLSI, 2012). The plates were incubated for another 4 h. The absorbance of XTT-XTT labeling mixture were added to each well and then the plates were incubated at 37°C for 2 h. The absorbance of the water and methanol extracts of *F. carica* were measured using a microplate reader (Epoch, USA) at 450 nm against the control (the same cells without treatment). The Tukey test was used as the post-hoc test. P values less than or equal to 0.05 were considered to be statistically significant. The 50% inhibitory concentrations of the extract and reference compounds were calculated through an extract dose-response curve in GraphPad Software (San Diego, CA, USA).

**Cell viability assay**

The antiproliferative activity of the *F. carica* leaf extracts was evaluated by XTT colorimetric assay against the MDA-MB-231 and L929 cells. Extracts were dissolved in DMSO and diluted in DMEM prior to treatment. Initially, cancer and control cells were seeded at a density of 5×10⁴ cells per well in 96-well culture plates in 100 µL of culture medium and were allowed to attach overnight before treatment. The next day, these cells were treated with serial concentrations (0.0625, 0.125, 0.25, 0.5, 1 mg/mL) of *F. carica* for 24 h. Furthermore, nontreated cells and cells treated with DMSO (0.5%) were used as negative controls and solvent controls, respectively. After that, the treatment medium was removed and wells were washed twice with 200 µL of PBS. At the end of these periods, for determination of living cells, 100 µL of DMEM without phenol red and 50 µL of XTT labeling mixture were added to each well and then the plates were incubated for another 4 h. The absorbance of XTT-formazan was measured using a microplate reader (Epoch, USA) at 450 nm against the control (the same cells without any treatment). All experiments were performed in three independent experiments and cell viability was expressed in % related to the control (100% viability).

**Statistical analysis**

Data obtained from *in vitro* antioxidant and antidiabetic activity were expressed as the mean ± SD. Cytotoxicity results were evaluated statistically using one-way analysis of variance (ANOVA) at 95% confidence levels for multiple comparisons. The Tukey test was used as the post-hoc test. P values less than or equal to 0.05 were considered to be statistically significant. The 50% inhibitory concentrations of the extract and reference compounds were calculated through an extract dose-response curve in GraphPad Software (San Diego, CA, USA).

**RESULTS AND DISCUSSION**

**GC-MS analysis of the water and methanol extracts of *F. carica***

The chemical compositions of the water and methanol extracts of *F. carica* leaves were studied using GC-MS and the results are shown in Table 1. According to the GC-MS results, more different components were obtained in the methanol extract than in the aqueous extract of *F. carica*. Namely, six and 28 different compounds were determined in the water and methanol extracts, respectively. While the most abundant components are benzene, methoxy-(3.32%), 4-methyl-1,4-heptadiene (6.85%), 1-pentene, 2,3-dimethyl-(2.72%) for the water extract, they were 2H-furo[2,3-H]-1-benzopyran-2-one (53.64%), bergapten (19.27%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-(4.05%) for the methanol extract.
When the extracts of the *F. carica* leaves were compared, we can see that the solubility of the methanol extracts was much greater than that of the water extracts, because the number of components is much higher in the methanol extracts. However, when we compare the enzyme inhibition activities, the water extracts showed higher inhibition activities than the methanol extracts (Table 2). This is most likely caused by the water extracts' components. It is also interesting that almost none of these components are present in the methanol extract. Likewise, in the study conducted by Konyalıoğlu, the amount of alpha-tocopherol in fig leaves was determined by HPLC and correlated with antioxidant activity. In our study, GC-MS analysis of the *F. carica* leaves shows that the antioxidant vitamin alpha-tocopherol (vitamin E) was found in the methanol extract.

### In vitro antioxidant activity

#### In vitro radical scavenging activity

In some physiopathologic circumstances, there is excessive production of free radicals, leading to the occurrence of oxidative stress. This later is related to the appearance of many diseases including Alzheimer's diseases, cardiovascular disease, and cancer. Natural antioxidants inhibit their activity by different mechanisms such as scavenging of reactive oxygen species, metal chelating, activation of antioxidant enzymes, and inhibition of oxidase. Therefore, it is necessary to use different methods to evaluate the antioxidant activity of extracts in plants. Previous studies have shown that the fig of *F. carica* has antioxidant activity. In our study, leaf extract of *F. carica* scavenged DPPH and ABTS radicals in a concentration-dependent manner. As shown in Figure 1a and 1b, the IC$_{50}$ of ABTS radical scavenging activity of the methanol and water extract was 559.39 µg/mL and 428.51 µg/mL, while DPPH scavenging activity was 1.45 mg/mL and 1.83 mg/mL, respectively.

The total phenolics (mg GAE/g of sample) and flavonoid (mg CE/g of sample) in the different extract of the *F. carica* leaves are exhibited in Figure 1c. The methanol extract (16.11 mg GAE/g) exhibited higher phenolic contents as compared to the water extract (6.29 mg GAE/g), while the total flavonoid content was almost the same as that of methanol (11.29 mg CE/g) and water (11.06 mg CE/g) extract. The phenolic compounds in fig leaves were quantitatively determined using HPLC-DAD by

### Table 1. Chemical components of the water and methanol extracts from *F. carica*

| Chemical components | RT (min) | Water (%) | Methanol (%) |
|---------------------|---------|-----------|--------------|
| 2-Furanmethanol (CAS) | 6.692   | ---       | 0.36         |
| 2-Cyclopentene-1,4-dione | 7.493   | ---       | 0.10         |
| Benzene, methoxy- | 8.654   | 3.32      | ---          |
| 1,2-Cyclopentanedione | 8.849   | ---       | 0.31         |
| Phenol (CAS) | 11.298  | -0.02     | 0.14         |
| 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 16.677  | ---       | 0.30         |
| 4-Methyl-1,4-heptadiene | 17.031  | 6.85      | ---          |
| 1-Pentene, 2,3-dimethyl- | 18.576  | 2.72      | ---          |
| 2-Furancarboxaldehyde, 5-(hydroxymethyl)- | 19.274  | ---       | 0.01         |
| 5-Acetyl-2-furanmethanol | 20.545  | 11.3      | ---          |
| Benzoic acid, 3-hydroxy-, methyl ester (CAS) | 26.770  | 1.47      | ---          |
| Cyclododecane | 28.218  | ---       | 0.66         |
| 2,7-Naphthalenediol (CAS) | 28.630  | ---       | 0.82         |
| Phenol, 2,4-bis(1-methyl)- | 29.288  | ---       | 0.64         |
| 1H-Imidazole, 4-methyl-5-nitro- | 30.827  | ---       | 0.10         |
| 6,7-Dimethoxyquinoloxaline | 31.931  | ---       | 0.10         |
| acrylolic acid dodecanoyl ester | 33.436  | ---       | 0.69         |
| (-)-Loliolide | 35.084  | ---       | 0.48         |
| 2H-Furo[2,3-H]-1-benzopyran-2-one | 36.280  | ---       | 53.64        |
| 7H-Furo[3,2-g]-1benzopyran-7-one | 36.649  | ---       | 0.01         |
| Hexadecanoic acid, methyl ester | 37.705  | ---       | 0.66         |
| Hexadecanoic acid (CAS) | 38.357  | ---       | 2.30         |
| Bergapten | 40.114  | ---       | 19.27        |
| 9,12,15-Octadecatrienoic acid, methyl ester (CAS) | 40.526  | ---       | 0.80         |
| 7-Oxabicyclo[4.1.0]heptane, 1,5-dimethyl- | 40.709  | ---       | 1.40         |
| Heptadecanoic acid, 16-methyl-, methyl ester | 40.886  | ---       | 0.10         |
| 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 41.207  | ---       | 4.05         |
| Methyl (Z)-5,11,14,17-eicosatetraenoate | 41.424  | ---       | 0.10         |
| Ferruginol | 43.970  | ---       | 0.28         |
| 4,8,12,16-Tetramethylheptadecan-4-olide | 44.176  | ---       | 0.11         |
| Isosteviol methyl ester | 45.899  | ---       | 0.38         |
| Docosa-2,6,10,14,18-pentaen-22-al, 2,6,10,15,18-pentamethyl-, alltrans | 51.272  | ---       | 1.07         |
| Vitamin E | 55.563  | ---       | 0.35         |

### Table 2. Enzyme inhibitory activity (%) of the water and methanol extracts from *F. carica* leaves (at 2 mg/mL concentrations)

| Extracts | AChE | BChE | α-glucosidase | α-amylase |
|----------|------|------|---------------|----------|
| Methanol | 53.33±3.21 | 57.97±5.61 | 64.93±1.09 | 67.32±2.46 |
| Water | 62.88±3.65 | 73.02±4.28 | 69.56±0.61 | 69.08±6.05 |

### Reference drugs

- **Galantamine hydrobromide**: 93.87±0.56, 89.89±0.01
- **Acarbose**: 57.56±0.52, 58.40±0.63

AChE: Acetylcholinesterase, BChE: Butyrylcholinesterase
Teixeira et al.33 We also achieved similar results using a different method in our study. In another study, by Ali et al.,34 it was shown that antioxidant and anti-inflammatory activities of fig leaves are associated with flavonoids and phenolic compounds found in the leaves.

It is well known that the ferrous and cupric ions stimulate lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton reaction. Therefore, metal chelating agents play an important role in terms of retarding the radical degradation by reducing the concentration of transition metal.35 According to our results, water extracts exhibited better iron chelating activity than methanol extract (Figure 1e). In the FRAP assay, the reductants (antioxidants) present in the extract reduce a Fe\textsuperscript{3+}–TPTZ complex to form blue Fe\textsuperscript{2+}–TPTZ. The change in absorbance at 593 nm is proportional to the FRAP value of the antioxidants in the sample.36 The results of the FRAP assay are given in Figure 1d. In this assay, the higher activity was noted for methanol extract than water extract at higher concentration, but the ferric reducing power was the same at the lower concentration.

![Figure 1. In vitro antioxidant activity of the methanol and water extracts from F. carica leaves; a) DPPH radical scavenging activity; b) ABTS radical scavenging activity; c) total phenol and total flavonoid contents; d) ferric reducing power as FeSO\textsubscript{4} equivalent; e) iron chelating activity.](image-url)
**AChE and BChE inhibition activity**

The methanol and water extracts prepared from *F. carica* leaves were evaluated for their inhibitory effects against AChE and BChE, which are Alzheimer’s disease-related enzymes. The water extract exhibited stronger activity and showed 63% and 73% inhibition of AChE and BChE, which was lower than the standard drug galantamine (with 93% and 90% inhibition) at the same concentration (Table 2). According to the report by Ahmad et al., the n-butanol fractions displayed the best anti-AChE activity, while ethyl acetate soluble fraction demonstrated the best anti-BChE activity among different solvent fractions of *F. carica* fruit. In the study by Orhan et al., the n-hexane and acetone extracts of leaves exhibited notable inhibition activity against both AChE and BChE. However, in our study, the aqueous extract was found to be more active than the methanol in terms of these two enzyme inhibitions. This may be due to the more polar compounds present in the aqueous extract active against AChE and BChE enzyme inhibition.

**In vitro α-glucosidase and α-amylase enzyme inhibition activity**

It is known that α-amilase and α-glucosidase are enzymes that catalyze the hydrolysis of polysaccharides and disaccharides to monosaccharides. The inhibition of these two enzymes hinders the rapid uptake of blood glucose levels by delaying the digestion of carbohydrates. The results of the inhibitory activity of the *F. carica* leaf methanol and water extracts against α-glucosidase and α-amylase enzyme are presented in Table 2. When compared to each other, the water extract (69.56% and 69.08%) was found to be higher than the methanol extract (64.93% and 67.32%) in inhibiting α-glucosidase and α-amylase enzyme activity, and both extracts were found to be potential inhibitors against α-glucosidase and α-amylase compared with the standard antidiabetic drug acarbose (57.56% and 58.4%) at the same concentration (2 mg/mL). In a recent study, similar antidiabetic activities were reported for the ethyl acetate and ethanol extracts of *F. carica* fruit. In another study, the ethyl acetate extract of *F. carica* leaves showed antidiabetic activity by stimulating insulin production from regenerated pancreas beta cells. Similar results were reported for the water and methanol extracts of *F. carica* leaves in our study.

**Antimicrobial activity**

The antimicrobial activities of *F. carica* methanol and water extracts against *S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *C. albicans* were detected using the microdilution technique at the concentration range 0.156 to 2.5 mg/mL (Table 3). It has been reported that the antimicrobial activity of plant extracts was evaluated as significant with MIC value less than or equal to 0.1 mg/mL, moderate with 0.1< MIC ≤0.625 mg/mL, and weak with MIC value greater than 0.625 mg/mL. According to these criteria, the methanol extract of *F. carica* showed moderate antimicrobial activities against *E. coli* (0.625 mg/mL) and *S. aureus* (0.156 mg/mL) and weak antimicrobial activity against the other bacteria and *C. albicans* (≥2.5 mg/mL). Similarly, the water extract of *F. carica* displayed moderate antimicrobial activity against *S. aureus* (0.625 mg/mL) and weak antimicrobial activity against the other bacteria and *C. albicans* (≥2.5 mg/mL).

*F. carica* methanol extract has been studied against various bacteria and showed moderate to strong antibacterial activity. In an *in vitro* study, Jeong et al. reported that *F. carica* methanol extract had strong antibacterial activity on oral bacteria. In another study, Keskin et al. investigated the antimicrobial activity of different extracts of *F. carica*. Their study reported that the MIC values of the methanol and aqueous extracts of *F. carica* against bacteria and *C. albicans* were MIC 25-400 μg/mL and MIC 200-400 μg/mL, respectively. In the present study, *E. coli* and *S. aureus* were more susceptible to the methanol extract. Our results revealed that the methanol and water extracts of *F. carica* exhibited weak antimicrobial effects against other bacteria and *C. albicans*.

**Cell viability**

The XTT cell proliferation assay was used to evaluate the antiproliferative effects of the water and methanolic extracts of *F. carica* on MDA-MB-231 and L929 cell lines. As shown in

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**Table 3. Antimicrobial activity results of *F. carica* methanol and water extracts**

| Microorganisms and MIC values (mg/mL) | E. coli | S. aureus | P. aeruginosa | E. faecalis | K. pneumoniae | C. albicans |
|--------------------------------------|--------|----------|-------------|------------|---------------|-----------|
| Methanol                             | 0.625  | 0.156    | >2.5       | >2.5       | >2.5          | 2.5       |
| Water                                | 2.5    | 0.625    | >2.5       | >2.5       | >2.5          | 2.5       |

MIC: Minimum inhibitory concentration
Figure 2, the methanol extract at all concentrations significantly inhibited MDA-MB-231 cell proliferation (p<0.05) in a dose-dependent manner (IC_{50}=0.081 mg/mL). On the other hand, concentration of 1 mg/mL of the water extract moderately decreased the cell viability (IC_{50}=1 mg/mL) (p<0.05). However, neither extract exhibited any significant cytotoxicity on the L929 cell line in the concentrations range (1-0.0625 mg/mL).

Our cytotoxicity results clearly indicated that the methanol extract is more toxic than the water extract of *F. carica*. This may be due to the fact that the methanol extract has richer active ingredients than the water extract, as shown in Table 1. Additionally, the anticancer effects may be associated with antioxidant features due to its polyphenolic components quantity (Figure 1). To the best of our knowledge, this is the first study of the anticancer effect of fig leaf extracts on MDA-MB-231. However, different parts of *F. carica* and different extracts of fig leaf have already been found to be cytotoxic on various cancer cells such as the stomach and cervix.6,44

**CONCLUSION**

Overall, in this study, the components and antioxidant, antimicrobial, anticancer, enzyme inhibition, and antidiabetic effects of *F. carica* leaf methanol and water extracts were investigated. Despite the several antioxidant activities of *F. carica* leaves, to the best of our knowledge there are no reports on the comparative study of extracts with different polarity as well as other antioxidant methods such as iron chelating and ferric reducing power. Our results indicated that especially the methanol extract has strong anticancer, antioxidant, and antidiabetic activities. There is a correlation between anticancer and antioxidant activity and total phenolic content. Moreover, the richer chemical content of the methanol extract may be associated with higher biological activity. Consequently, the methanolic extract of the leaf of *F. carica* may be considered a potential therapeutic agent in cancer and diabetes mellitus. However, further studies, particularly in vivo experiments, are needed to verify these effects.

**Conflicts of interest:** No conflict of interest was declared by the authors.

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