Effect of storage conditions on the physicochemical properties, polyphenolic content, and biological activities of fig leaves (*Ficus carica* L.)

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Abstract. Fig tree leaves (*Ficus carica* L.) are a rich source of biologically active compounds and possess both nutritional value and therapeutic potential. This study compared the physicochemical properties, polyphenolic content, and biological activities of fig leaves processed and stored under different conditions: air-drying and freezing. Dried leaves (DL) exhibited higher protein (17.06 %) and carbohydrate (11.4 %), contents than the frozen leaves (FL) - 4.14 % and 5.93 %. DL extract exhibited higher contents of total polyphenols and flavonoids (24.53 mg GAE/g dw and 0.83 mg QE/g dw) than FL extract (1.80 mg GAE/g dw and 0.07 mg QE/g dw). DL showed greater antioxidant activity (DPPH: 183.81 vs. 8.38; FRAP: 193.60 vs. 13.81 mM TE/g dw). Antimicrobial tests revealed higher inhibitory activity (inhibition zones ≥ 12 mm) of DL extract against *Bacillus subtilis*, *Bacillus amyloliquefaciens, Bacillus cereus, Enterococcus faecalis*, *Micrococcus luteus*, *Salmonella enteritidis*, *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, while FL extract was less active. Both dried and frozen fig leaves exhibited notable biological activities, with DL showing superior antioxidant and antimicrobial potential. Consequently, drying was the more suitable storage method, better preserving the bioactivity of fig leaves and enhancing their functional and therapeutic potential.

1 Introduction

The fig (*Ficus carica* L.) is a shrub or small tree belonging to the Moraceae family, which is among the earliest domesticated fruit trees and has a long history of use for both nutritional and medicinal purposes across the Mediterranean, the Middle East, and other regions of the world. Its fruits can be consumed fresh, dried, or processed (jams and syrups), while its leaves can be used to prepare teas and infusions, and as flavoring agents. Whereas the fruits of *F. carica* are well known for their nutritional value—being rich in soluble carbohydrates and dietary fibers, vitamins, and minerals—its leaves have recently attracted scientific attention due to their promising bioactive potential [1-4]. Some recent studies have also revealed the potential application of fig leaves as natural food preservatives [5, 6]. The traditional uses of fig leaves are increasingly supported by numerous scientific studies investigating the phytochemical profile, antioxidant properties, as well as various biological activities of their extracts. In ethnomedicine, fig leaves have been used for the treatment of gastrointestinal diseases (colic, indigestion, diarrhea, loss of appetite), respiratory disorders (sore throat, cough, bronchitis), dermatological conditions (furuncles, psoriasis, vitiligo), metabolic imbalances (hyperlipidemia and diabetes), cardiovascular disorders, cancer, degenerative diseases, as well as for their strong anti-inflammatory properties [1, 2, 7-9].

The studies on phytochemical composition of fig leaves have been demonstrated to contain substantial amounts of phenolic acids, flavonoids, tannins, organic acids, carbohydrates, fatty acids (including polyunsaturated fatty acids), as well as tocopherols (vitamin E isoforms). Polyphenols such as caffeoylmalic acid, rutin, and isoschaftoside are predominant in the leaves of several fig cultivars, while the furanocoumarins psoralen and bergapten are the major phenolic compounds identified in others [3, 10]. In addition, recent comparative studies have shown that the levels of organic acids, tocopherols, and fatty acids vary significantly among fig cultivars, indicating genotype-dependent differences in their bioactive composition [2]. It should be noted that various external factors, including post-harvest processing and storage conditions, can influence both

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the concentration of bioactive compounds and the biological activity of fig leaves. For instance, fig leaves dried under different conditions exhibited varying antioxidant capacity; shade-dried leaves tend to preserve antioxidant and anti-inflammatory activities more effectively than those dried in an oven [11].

F. carica is considered a medicinal plant with great therapeutic potential. Phenolic compounds identified in fig leaves have been shown to exhibit strong antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, antispasmodic, acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE) inhibitory effects. Moreover, they can prevent the production of reactive oxygen species involved in the pathogenesis of major diseases such as cancer and diabetes mellitus, and may aid in relieving the symptoms of Alzheimer's disease [12]. There is also evidence that aqueous fig leaf extract can influence gene expression relevant to the inflammatory process *in vitro*, through downregulation of vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), interleukin 1-α (IL-1α), and 5 α-reductase type II (SRD5A2) in a human keratinocyte cell line (HaCaT), suggesting a role in the modulation of inflammation and the restoration of skin homeostasis [13].

In recent years, there has been a growing interest in the utilization of agricultural waste as a part of global efforts toward sustainable resource management. According to recent agricultural statistics, global fig fruit production was estimated at 1153 tons in 2019. Despite this, fig leaves remain largely underutilized, leading to the accumulation of significant amounts of agricultural bio-waste. The integration of agri-food by-products into the value chain, aimed at reducing waste and promoting sustainable practices throughout the production process, remains a key challenge within the framework of the circular economy. As a bio-waste generated from fig cultivation, fig leaves hold considerable potential as a source of biologically active compounds applicable across various industries. These compounds could serve as natural substitutes for synthetic additives in the food industry, while simultaneously improving the physical and sensory properties of the final products [2]. In this regard, the effective valorization of fig leaves critically depends on proper harvesting, drying, or other storage methods in order to preserve their biologically active compounds and maintain their effectiveness [11, 14].

Therefore, the present study aimed to evaluate and compare the physicochemical properties, polyphenolic content, and biological activities of fig leaves stored under two different conditions, namely air-drying and freezing, and to assess which method is more suitable for their storage. Understanding these relationships may facilitate the valorization of fig leaf biomass, commonly considered a by-product or waste, for potential use in nutraceutical, pharmaceutical, or cosmetic applications.

2 Materials and methods

2.1 Materials

2.1.1 Plant material

In the present study, fig leaves (*Ficus carica* L.) were collected in June 2025 from a tree cultivated in a private yard in the village of Bolyartsi, Plovdiv district, Bulgaria (42°07′N, 24°96′E; altitude 177 m). After harvesting, the fresh leaves were washed with tap water and divided into two equal portions.

One portion was frozen at −15 °C and stored for 15 days prior to analysis. This temperature was chosen based on standard laboratory equipment availability and prior evidence indicating adequate preservation of phytochemicals for the study duration, with all samples handled consistently.

The other portion was air-dried under controlled ambient conditions: temperature 22–25 °C, relative humidity approximately 40–60 %, in shaded and ventilated areas to ensure uniform drying. Leaves were arranged in a single layer with standardized thickness of ~0.5 mm to optimize air circulation. Drying continued until a residual moisture content of 10 % was achieved (approximately 15 days).

Both frozen and dried plant materials were ground prior to analysis (Fig. 1). Analyses were performed immediately after drying or thawing, within 15 days of collection, to minimize degradation and ensure comparability between treatments.

Fig. 1. Ground fig leaf material: frozen (left) and dried (right).

2.1.2 Test microorganisms

Twenty-four microorganisms including seven Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus amyloliquefaciens* 4BCL-YT, *B. cereus* NCTC 11145, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* NBIMCC 8632, *Enterococcus faecalis* ATCC 19433, and *Micrococcus luteus* 2YC-YT), seven Gram-negative bacteria (*Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* NBIMCC 1672, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Proteus mirabilis* 56/10, and *Pseudomonas aeruginosa* ATCC 9027), two yeasts (*Candida albicans* NBIMCC 74 and

Saccharomyces cerevisiae ATCC 9763) and eight fungi *Aspergillus niger* ATCC 1015, *Aspergillus flavus*, *Aspergillus ochraceus*, *Penicillium chrysogenum*, *Fusarium moniliforme* ATCC 38932, *Fusarium oxysporum*, *Rhizopus* sp., and *Mucor* sp.) were selected for the antimicrobial activity test.

2.1.3 Culture media

Luria-Bertani agar medium with glucose (LBG agar)

LBG agar was used for the cultivation of test bacteria. A quantity of 50 g of LBG-solid substance mixture was dissolved in 1 L of deionized water, pH 7.5 ± 0.2.

Malt extract agar (MEA)

MEA was used for the cultivation of test yeasts and fungi. A quantity of 50 g of the MEA-solid substance mixture was dissolved in 1 L of deionized water, pH 5.4 ± 0.2.

Both culture media were prepared in accordance with the manufacturer's instructions (Scharlab SL, Spain) and autoclaved at 121 °C for 20 min (liquid phase) prior to use.

2.2 Methods

2.2.1 Extracts preparation

Using a blender, the dried and frozen fig leaves were initially ground. Each powdered sample (4 g) was then macerated with 40 ml of 70% ethanol (Sigma-Aldrich, Merck, Germany). The samples were kept at room temperature in the dark for 72 h after being stirred for 10–15 s using a V-1 vortex mixer (Biosan, Latvia). After filtration through filter paper, the extracts were stored at 4 °C until analysis.

For the antimicrobial activity test, methanolic extracts of the same concentration were prepared, as methanol had previously been determined not to possess inhibitory effect on the test microorganisms used [15]. For the HPLC quantification of organic acids and carbohydrates, aqueous extracts of the same concentration were prepared, as water-based solvents are more compatible with the chromatographic column and mobile phase.

2.2.2 Moisture content

Determination of moisture content was performed using a KERN DAB 100-3 moisture balance analyser (Kern&Sohn GmbH, Germany) by heating a 1 g sample of ground plant material at 110°C to a constant weight. The moisture content (%) was automatically recorded by the analyser based on the difference between the initial weight and the weight after drying of the sample [16].

2.2.3 Protein content

The protein content was determined by the nitrogen content of the samples according to the Kjeldahl nitrogen determination method (AOAC) using a BUCHI K-365 Kjel Line automatic system (Hanon, Germany) [16].

2.2.4 Ash and carbohydrates

The ash and carbohydrate contents were assessed in accordance with the Bulgarian State Standards [17,18].

2.2.5 Total phenolic content

The total phenolic content (TPC) was determined using a Folin-Ciocalteu reagent by the method of Ivanov et al. [19]. The results were expressed as mg equivalent of gallic acid (GAE)/g of dry weight (dw) sample.

2.2.6 Total flavonoid content

The total flavonoid content (TFC) was evaluated according to Ivanov et al. [19]. The results were expressed as mg quercetin equivalents (QE)/g of dw sample.

2.2.7 Antioxidant activity

DPPH radical scavenging assay

The antioxidant activity using the DPPH reagent (2,2-diphenyl-1-picrylhydrazyl) was evaluated according to Ivanov et al. [19]. The results were expressed as mM Trolox equivalents (TE)/g of dw sample.

Ferric-reducing antioxidant power (FRAP) assay

The antioxidant activity using the FRAP reagent was assessed as previously described by Ivanov et al. [19]. The results were expressed as mM TE/g of dw sample.

Calibration curves were prepared using gallic acid for TPC, quercetin for TFC, and Trolox for DPPH and FRAP assays. All measurements were performed with appropriate reagent blanks, and absorbance values were corrected accordingly. Results were normalized to dry weight based on the amount of dry plant material used for extraction and the final extract volume.

2.2.8 High-performance liquid chromatography (HPLC) analysis of phenolic compounds

The individual phenolic acids of fig leaf extracts were determined using an HPLC unit Elite LaChrome (VWR™ Hitachi, Tokyo, Japan) equipped with a diode array detector (DAD) as previously described. The results were expressed as μg/g dw.

The individual flavonoids were detected using Waters 1525 Binary Pump HPLC system (Waters, Milford, MA, USA) equipped with Waters 2484 dual Absorbance Detector (Waters, Milford, MA, USA) and Supelco Discovery HS C18 column (5 μm, 25 cm × 4.6 mm)

operating under the control of Breeze 3.30 software. The results were expressed as μg/g dw.

The quercetin glycosides were analyzed using the same HPLC system by gradients of 2% (v/v) acetic acid (Sigma) (solvent A) and acetonitrile (Sigma) (solvent B). Detection was carried out at 370 nm as previously described. The results were expressed as μg/g dw [20].

2.2.9 HPLC analysis of organic acids

The organic acid analysis was performed on the Elite LaChrome (Hitachi, Tokyo, Japan) HPLC system equipped with DAD as previously described [20]. The separation was conducted on a Discovery® SH C18 column (25 cm × 4.6 mm, 5 μm) (Supelco) at 30 °C, and isocratic elution with a mobile phase consisting of 25 mM KH_2PO_4 (pH 2.4 with H_3PO_4). L-(+)-ascorbic acid was detected at 244 nm, while citric, fumaric and L-malic acids were detected at 210 nm. The results were expressed as mg/g dw.

2.2.10 HPLC analysis of carbohydrates

The carbohydrate analysis was performed on the Elite LaChrome (Hitachi, Tokyo, Japan) HPLC system equipped with a refractive index detector (RID) Chromaster 5450 (WVR, Hitachi, Tokyo, Japan). The HPLC separation was carried out on a Shodex® Sugar SP0810 column with Pb^{2+} (300 mm × 8.0 mm) and a guard column Shodex SP-G (6 mm × 50 mm, 5 μm) at a temperature of 80 °C, a temperature of RID 35 °C and distilled water as a mobile-phase [20]. The results were expressed as mg/g dw.

All quantitative results were calculated and expressed on a dry matter basis for both frozen and air-dried fig leaf samples, thus accounting for differences in water content between the two materials. Therefore, the comparison between samples was not affected by the initial moisture differences.

2.2.11 Antimicrobial activity

The antimicrobial activity of the methanolic fig leaf extracts was determined by the standard agar well diffusion method [21]. After the incubation (24/48 h), the inhibitory effect was determined by measuring the inhibition zones (IZs) diameter around the wells. The antimicrobial activity was assessed as follows: high (IZ diameter ≥ 18 mm); moderate (IZ diameter 12 - 18 mm); low (IZ ≤ 12 mm). As positive controls, the antibiotics Cefotaxime (against bacteria) and Nystatin (against yeasts and fungi) at concentration of 10 mg/ml were used.

2.2.12 Statistical analysis

All experiments were performed in triplicates. Data were processed with MS Office Excel 2010 software using statistical functions to determine the standard deviation (±SD) and maximum estimation error at significance levels $p \leq 0.05$. One-way analysis of variance (ANOVA) was performed using the Statgraphics Centurion statistical program, version XVI, 2009 (Stat Point Technologies, Inc., Warrenton, VA, USA) [20].

3 Results and discussions

3.1 Physicochemical characteristics

As presented in Table 1, the air-dried fig leaves (DL) exhibited lower moisture and higher ash contents compared to the frozen leaves (FL). Regarding the protein and carbohydrate contents, DL material showed considerably higher values than FL. These findings indicated that the air-drying process is more effective than freezing in preserving the nutritional components of fig leaves, suggesting that it is a superior method for their storage and subsequent utilization.

Table 1. Physicochemical characteristics of fig leaves

Parameter	Fig leaves	
	Frozen	***Dried***
Moisture, %	75.25 ± 0.66[a]	10.49 ± 0.11[b]
Ash, %	5.21 ± 0.16[a]	12.24 ± 0.02[b]
Carbohydrates, % dw	5.93 ± 0.06[a]	11.40 ± 0.40[b]
Proteins, % dw	4.14 ± 0.28[a]	17.06 ± 0.78[b]

[a-b] Values within the same row with different superscript letters differ significantly ($p < 0.05$)

A study on the proximate composition of fresh *F. carica* leaves demonstrated that they contained 62.6 % moisture, 4.3 % ash, 6.3 % proteins, 0.91 % lipids, and 19.49 % carbohydrates [22]. In contrast, the proximate analysis conducted by Osowe et al. [23] demonstrated that fig leaves powder had 8.69 % moisture, 12.97 % ash, 15.76 % protein, and 4.59 % lipid contents. These findings were in agreement with our results, indicating a consistent chemical composition among the different studies and further emphasizing the nutritional value of *F. carica* leaves as a potential source of bioactive nutrients. Investigating the nutritional composition of freeze-dried fig leaves from Saudi Arabia, Ghazi et al. [24] reported that the protein content of small and big leaves ranged from 4.6 to 5.1 %, while their fat content was 0.9 and 1.3 %, respectively. Both types of leaves exhibited similar total ash (4.2 and 4.4 %, respectively) and carbohydrate (16.8 and 17.3 %, respectively) contents.

3.2 Total phenolic content, total flavonoid content and antioxidant activity

The results presented in Table 2 indicated a decrease in the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity values (as determined by the DPPH and FRAP assays) of the frozen fig leaves. Similar to the physicochemical parameters, this trend was likely due to the degradation of biologically active compounds during the freezing process at –15 °C. Consequently, air-drying of fig leaves appeared to be a

more effective technological approach for preserving the levels of bioactive compounds.

Table 2. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of ethanolic fig leaf extracts

Parameter	Fig leaves	
	Frozen	***Dried***
TPC, mg GAE/g dw	1.80 ± 0.01[a]	24.53 ± 0.30[b]
TFC, mg QE/g dw	0.07 ± 0.01[a]	0.83 ± 0.11[b]
DPPH, mM TE/g dw	8.38 ± 0.22[a]	183.81 ± 2.25[b]
FRAP, mM TE/g dw	13.81 ± 0.50[a]	193.60 ± 1.70[b]

[a-b] Values within the same row with different superscript letters differ significantly ($p < 0.05$).

The observed differences between air-dried leaves (DL) and frozen leaves (FL) in physicochemical parameters and phytochemical content (Tables 1 and 2) can be attributed to differences in moisture content and post-harvest metabolic responses, rather than simple concentration effects. Air drying rapidly reduces tissue moisture, limiting nutrient degradation while simultaneously triggering stress-related metabolic processes that may enhance the synthesis or release of phenolic compounds and other bioactive substances, as previously reported. Partial hydrolysis of carbohydrates and starch during drying may also contribute to the increased sugar content observed in DL samples [25].

In contrast, freezing at −15 °C does not fully suppress enzymatic activity, particularly during freezing and thawing. Residual enzyme activity, combined with membrane disruption caused by ice crystal formation, may lead to the substantial reductions in protein and carbohydrate contents observed in FL samples, as well as to the decrease in TPC, TFC, and antioxidant activity, as measured by the DPPH and FRAP assays. These reductions were probably due to the partial degradation or reduced extractability of bioactive compounds during the freeze–thaw process [26]. Consequently, air drying appears to be a more effective approach for preserving the nutritional and bioactive composition of fig leaves.

According to the literature, the polyphenolic content and antioxidant potential of fig leaves can vary widely depending on the cultivar, environmental conditions, and post-harvest processing. Ghazi et al. [24] reported that higher TPC values were found in the aqueous extracts of big and small fig leaves from Saudi Arabia (907.02 and 629.78 mg GAE/100 g, respectively), whereas the methanolic extracts showed lower values of 412.37 and 275.35 mg GAE/100 g, respectively. The same authors observed that the methanolic extract of big leaves exhibited the highest FRAP value (131.39 mmol Fe^{2+}/100 g), followed by the small-leaf extract (101.46 mmol Fe^{2+}/100 g). In the case of the aqueous extracts, the big leaves showed insignificantly higher FRAP activity (16.66 mmol Fe^{2+}/100 g) compared to the small ones (16.25 mmol Fe^{2+}/100 g). The results obtained by Mahmoudi et al. [9] for the methanolic leaf extracts of ten Algerian *F. carica* varieties demonstrated that the TPC values ranged from 42.89 to 58.70 mg GAE/g of dry extract (de), while the TFC values varied between 11.67 and 16.21 mg QE/g de. The antioxidant capacity of the investigated extracts, determined by the DPPH radical scavenging assay and expressed as IC_{50}, ranged from 659.97 to 1119.59 µg/ml. Tikent et al. [27] reported that the leaves of four different fig varieties from Eastern Morocco showed TPC values ranging from 58.1 to 62.6 mg GAE/g dw, while TFC values ranged between 24.5 and 26.2 mg QE/g dw. DPPH scavenging capacity, expressed as IC_{50}, ranged between 0.7 and 1.3 mg/ml, whereas antioxidant capacity assessed by the ABTS scavenging assay ranged from 87.1 to 112.2 TE µmol/ml.

3.3 HPLC analysis of phenolic compounds

In order to determine the polyphenolic profile of fig leaf extracts, an HPLC analysis was performed. As shown in Table 3, thirteen phenolic compounds were identified in the dried fig leaf extract, including eight phenolic acids, four flavonoids, and one quercetin glycoside. Among the phenolic acids, rosmarinic acid was predominant, followed by salicylic, ferulic, and vanillic acids. Among the flavonoids, (–)-epicatechin was predominant, followed by (+)-catechin. In contrast, in the frozen leaf extract, only three phenolic acids (p-coumaric, ferulic, and salicylic) were detected, while the flavonoids quercetin and kaempferol were under the limit of quantification (ULOQ). These results confirmed the negative impact of freezing at –15 °C on the content of biologically active compounds.

Table 3. Quantitative HPLC analysis of phenolic compounds in ethanolic fig leaf extracts

Phenolic compound, µg/g dw	Fig leaves	
	Frozen	***Dried***
Phenolic acids		
Protocatehuic	-*	1.49 ± 0.05
Chlorogenic	-	26.11 ± 0.11
Vanillic	-	79.51 ± 0.35
Syringic	-	16.66 ± 0.18
***p*-Coumaric**	0.92 ± 0.03[a]	38.63 ± 0.21[b]
Ferulic	29.08 ± 0.14[a]	84.06 ± 0.51[b]
Salicylic	36.68 ± 0.20[a]	245.15 ± 1.59[b]
Rosmarinic	-	353.14 ± 2.06
Flavonoids		
(+)-Catechin	-	32.10 ± 0.25
(-)-Epicatechin	-	100.26 ± 0.79
Quercetin	ULOQ**	6.18 ± 0.12
Kaempferol	ULOQ	30.86 ± 0.44
Quercetin glycosides		
Rutin	-	28.62 ± 0.31

* "-" – not detected
** - Under the limit of quantification
[a-b] Values within the same row with different superscript letters differ significantly ($p < 0.05$)

A study conducted by Shiraishi et al. [2] on five fig cultivars from Portugal identified a total of thirteen phenolic compounds, including five phenolic acids (vanillic, caffeic, chlorogenic, and *p*-coumaric acid derivatives) and eight flavonoids (*C*-glycosylated derivatives of apigenin and luteolin, and *O*-glycosylated derivatives of quercetin). Using ultra-performance liquid chromatography coupled with mass spectrometry (UPLC–MS) analysis, Yahiaoui et al. [28] examined the phenolic profile of Algerian fig leaves and identified metabolites belonging to three classes: phenolic acids (chlorogenic, caffeic, and coumaric acids), flavonoids and flavonoid glycosides (rutin, isoquercetin, luteolin, quercetin, and kaempferol), and a furanocoumarin (psoralen). Phytochemical profiling by HPLC-UV of four Eastern Moroccan fig cultivars identified two phenolic acids (caffeic and trans-ferulic) and six flavonoids, including flavonoid glycosides (catechin, rutin, naringin, myricetin, kaempferol, and quercetin) [27].

Numerous studies have reported the physiological activities of phenolic compounds present in fig leaves. Chlorogenic acid is known for its antipyretic, anti-inflammatory, and analgesic properties. Caffeic acid (3,4-dihydroxycinnamic acid), a representative member of the hydroxycinnamic acid group, has demonstrated a wide range of beneficial biological effects, acting as a preventive agent against inflammatory processes, neurodegenerative disorders, and various cancers. *p*-coumaric acid has exhibited notable antidiabetic and antihyperlipidemic activities. Quercetin and quercetin glycosides are potent inhibitors of inflammation, through modulation of the expression of nitric oxide synthase (NOS) and C-reactive protein (CRP). Psoralen, classified as a furanocoumarin and abundantly present in fig leaves, exhibits strong biological activities. It has been reported to possess photosensitizing properties and is therefore used in the treatment of skin diseases such as psoriasis, neoplasms, and vitiligo [28].

3.4 HPLC analysis of organic acids and carbohydrates

As seen in Table 4, three organic acids (malic, ascorbic, and citric) and three sugars (sucrose, glucose, and fructose) were identified by HPLC. Citric acid was the predominant organic acid in both DL and FL extracts, while sucrose was the major sugar. However, the levels of these compounds were markedly lower in the FL extract, and ascorbic acid was not detected. These results indicated that the freezing process adversely affected the preservation of nutrients and bioactive compounds in fig leaves.

Table 4. Quantitative HPLC analysis of organic acids and carbohydrates in aqueous fig leaf extracts

Compound, mg/g dw	**Fig leaves**	
	Frozen	***Dried***
Organic acids		
Malic	2.25 ± 0.05^{a}	15.00 ± 0.24^{b}
Ascorbic	-*	3.00 ± 0.06
Citric	5.25 ± 0.09^{a}	45.00 ± 0.58^{b}
Carbohydrates		
Sucrose	18.75 ± 0.88^{a}	53.40 ± 0.95^{b}
Glucose	18.00 ± 0.65^{a}	32.04 ± 0.71^{b}
Fructose	3.00 ± 0.02^{a}	26.40 ± 0.22^{b}

* "-" – not detected
[a-b] Values within the same row with different superscript letters differ significantly ($p < 0.05$)

Using HPLC–IR analysis, Shiraishi et al. [2] studied the leaves of five *F. carica L.* cultivars from Portugal and identified five free sugars: sucrose (0.13–4.27 g/100 g dw), glucose (1.48–7.72 g/100 g dw), fructose (1.36–7.34 g/100 g dw), trehalose (0.61–1.13 g/100 g dw), and raffinose (0.26–0.87 g/100 g dw). The analysis of organic acids revealed the presence of oxalic (11.90–60.00 g/100 g dw), quinic (16.10–23.30 g/100 g dw), malic (2.54–55.80 g/100 g dw), and citric acids (21.80–27.60 g/100 g dw), whereas ascorbic, fumaric, and shikimic acids were found in trace amounts or were not detected. Another study on the leaves of two Portuguese *F. carica* varieties reported the presence of five organic acids: oxalic, malic, quinic, shikimic, and fumaric, with malic acid being the most abundant [4]. According to Ghazi et al. [24] the ascorbic acid content of small and big fig leaves ranged from 21.78 to 22.42 mg/100 g dw, which was lower compared to our results for DL. Significantly lower ascorbic acid content than that observed in our study was reported for four fig varieties originating from Eastern Morocco (2.3 – 8.2 mg/100 g dw) [27].

3.5 Antimicrobial activity

The results of the antimicrobial activity of frozen and dried fig leaf extracts are presented in Table 5.

Table 5. Antimicrobial activity of methanolic fig leaf extracts

Test microorganisms	**Diameter of inhibition zones, mm**		
	Fig leaf extracts (100 mg/ml)		**Controls (10 mg/ml)**
	Frozen	***Dried***	
B. subtilis	9 ± 0.00^{a}	13 ± 0.71^{b}	27 ± 0.00^{c}*
B. amyloliquefaciens	-***	12 ± 0.71^{a}	45 ± 0.71^{b}*
B. cereus	10 ± 0.71^{a}	13 ± 0.00^{b}	38 ± 0.00^{c}*
S. aureus	8 ± 0.00^{a}	10 ± 0.00^{b}	45 ± 1.41^{c}*
L. monocytogenes	-	10 ± 0.00^{a}	37 ± 0.00^{b}*
E. faecalis	-	17 ± 0.71	-*
M. luteus	8 ± 0.00^{a}	17 ± 1.41^{b}	32 ± 0.00^{c}*
S. enteritidis	13 ± 0.71^{a}	15 ± 0.00^{b}	40 ± 0.71^{c}*
S. typhimurium	-	8 ± 0.00^{a}	38 ± 0.00^{b}*
K. pneumoniae	-	10 ± 0.00^{a}	40 ± 0.00^{b}*

E. coli	10 ± 0.00^{a}	14 ± 0.00^{b}	30 ± 0.00^{c}*
P. vulgaris	8 ± 0.00^{a}	12 ± 0.00^{b}	45 ± 1.41^{c}*
P. mirabilis	-	17 ± 0.71^{a}	37 ± 0.71^{b}*
P. aeruginosa	10 ± 0.00^{a}	17 ± 0.71^{b}	20 ± 0.00^{c}*
C. albicans	-	-	12 ± 0.00**
S. cerevisiae	-	8 ± 0.00^{a}	17 ± 0.00^{b}**
A. niger	-	-	-**
A. ochraceus	-	-	-**
A. flavus	-	-	-**
P. chrysogenum	-	-	-**
F. moniliforme	-	8 ± 0.00^{a}	12 ± 0.00^{b}**
F. oxysporum	-	-	8 ± 0.00**
***Rhizopus* sp.**	-	8 ± 0.00^{a}	8 ± 0.00^{a}**
***Mucor* sp.**	-	-	-**

*- Cefotaxime; ** - Nystatin (10 mg/ml)
*** "-" – no inhibitory activity
[a-c] Values within the same row with different superscript letters differ significantly ($p < 0.05$)

Antimicrobial test revealed moderate inhibitory activity (IZs of 12–17 mm) of the dried fig leaf methanolic extract against *B. subtilis*, *B. amyloliquefaciens, B. cereus, E. faecalis*, *M. luteus*, *S. enteritidis*, *E. coli*, *P. vulgaris*, *P. mirabilis*, and *P. aeruginosa*, and low inhibitory activity (IZs <12 mm) against *S. aureus*, *L. monocytogenes*, *S. typhimurium*, *K. pneumoniae*, yeasts *S. cerevisiae*, and fungi *F. moniliforme* and *Rhizopus* sp. In contrast, the frozen fig leaf methanolic extract exhibited moderate inhibitory effect only on *S. enteritidis* and weak inhibitory effect on *B. subtilis*, *B. cereus, S. aureus*, *M. luteus*, *E. coli*, *P. vulgaris*, and *P. aeruginosa*. The solvent control (methanol) showed no antimicrobial activity (data not shown). The observed lower antimicrobial potential was probably due to the reduced phenolic content of the frozen fig leaves (FL), resulting from the adverse effect of storage at –15 °C on phenolic compounds, which are known for their antimicrobial properties. Compounds such as tannins, saponins, alkaloids, phenolic acids, flavonoids, quercetin glycosides, and phytosterols have been widely reported to exhibit strong antimicrobial activity [29].

A research by Keskin et al. [30] demonstrated that the ethanolic extract of dried fig leaves exhibited the highest antimicrobial activity compared to the methanolic and aqueous extracts. The ethanolic extract showed IZs with diameter ranging from 12 mm (against *K. pneumoniae* CCM 2318) to 28 mm (against *B. subtilis* ATCC 6633), indicating a broad-spectrum antimicrobial effect. In contrast, the methanolic extract showed moderate activity, with inhibition zones ranging from 9 mm (against *Aeromonas hydrophila* ATCC 19570) to 15 mm (against *B. subtilis* ATCC 6633). The aqueous extract displayed smallest inhibition zones (8–14 mm) and was inactive against three of the tested microorganisms (*S. typhimurium* CCM 583, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 10239). The analysis of the antimicrobial activity of hydroethanolic extracts from four fig cultivars from Eastern Morocco demonstrated that these extracts exhibited the strongest inhibitory effect against the yeast *C. albicans* (IZ = 18.6–20.4 mm). A comparatively lower, yet notable, inhibitory activity was observed against the bacterial strains *E. coli* (IZs = 12.5–12.9 mm), *P. aeruginosa* (IZs = 11.7–13.95 mm), *S. aureus* (IZs = 10.4–11.6 mm), and *B. subtilis* (IZs = 12.5–14.0 mm) [27]. Rostam et al. [31] reported lower antimicrobial activity compared to our findings. In their study, the methanolic fig leaf extract tested at various concentrations showed the highest effectiveness against *S. aureus* at 900 mg/ml (IZ = 9.0 mm) and *E. coli* at 700 mg/ml (IZ = 10.33 mm). Compared with our results, Belattar et al. [32] reported lower antimicrobial activity, as methanolic extracts (100 mg/ml) from dried leaves of ten Algerian fig cultivars exhibited IZs of 6–8.25 mm against *S. aureus* and *P. aeruginosa*, 6 mm against *E. coli*, and 7–15.75 mm against *K. pneumoniae*.

4 Conclusion

Fig leaves are a rich source of bioactive compounds, including polyphenols (phenolic acids, flavonoids, and quercetin glycosides), organic acids, and carbohydrates, which were confirmed by HPLC analyses and possess strong antioxidant potential and antimicrobial activities. These properties make fig leaves a promising natural ingredient for diverse applications in the food and pharmaceutical industries. Regarding their preservation, air-drying proved to be the more suitable technological approach, maintaining the biological activity and enhancing the functional and therapeutic potential of fig leaves.

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