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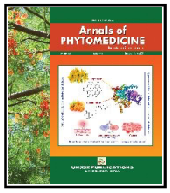

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Valorisation of *Ficus carica* L. leaves: Chemical characterization and bioactive potential

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Abstract

The fruit tree *Ficus carica* L. is among the earliest cultivated plant species. The present study aimed to evaluate the chemical composition and biological activity of methanolic extracts obtained from the mature, dried leaves of six Tunisian *F. carica* cultivars. Fatty acid profiles were determined by gas chromatography, while spectrophotometric methods were employed to quantify sugars, pigments, and phenolic compounds. Antioxidant capacity was assessed using two colorimetric assays: the ferric reducing power (RP) assay and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Among the cultivars analyzed, Bither Abiadh exhibited the highest chlorophyll content (41.72 µg/ml) and a comparatively elevated total sugar content (0.48 g/100 g dry weight). The Hemri cultivar, distinguished by its red fruit peel, demonstrated the greatest levels of total phenolics, o-diphenols, flavonoids, condensed tannins, and antioxidant activity. Saturated fatty acids constituted approximately 50% of the total fatty acid content, with palmitic acid identified as the predominant component. Polyunsaturated fatty acids represented 37% of the total fatty acids, with linoleic and linolenic acids as the major constituents. Antimicrobial activity assays against various bacterial strains revealed no significant inhibitory effects. Collectively, these findings highlight the potential of *F. carica* leaf extracts as a rich source of polyphenolic compounds with considerable antioxidant properties, underscoring their suitability for potential applications in the nutraceutical and functional food industries.

1. Introduction

Figs (*Ficus carica* L.), a deciduous species belonging to the Moraceae family, rank among the oldest cultivated fruit trees, alongside apples and grapes (Kislev *et al.,* 2006). They are widely distributed across diverse climatic zones and are particularly well-adapted to the Mediterranean region, including the varied environmental and soil conditions of Tunisia (Mars *et al.,* 1998). Despite their strong adaptability to the Tunisian climate, fig cultivation in the country largely remains traditional. Tunisia hosts a rich diversity of *F. carica* cultivars, many of which are conserved in germplasm collections across the country (Rhouma, 1996; Mars *et al.,* 1994).

Figs are a key component of the Mediterranean diet and can be eaten mostly fresh or dried. They are also processed into various food products such as jams, syrups, and fermented beverages (Mars *et al.,* 1998). Nutritionally, figs are recognized as a valuable source of vitamins, minerals, carbohydrates, and dietary fiber. They are low in fat and cholesterol while being rich in essential amino acids, contributing to their classification as a health-promoting food (Slatnar *et al.,* 2011; Veberic *et al.,* 2008; Solomon *et al.,* 2006). Numerous phenolic compounds have been found in this species, according to certain research (Teixeira *et al.,* 2006; Guarrera, 2005), and fig trees' fruits and branches are significant sources of fatty acids and phytosterols (Jeong and Lachance, 2001). Additionally noteworthy is its antioxidant capacity (Li *et al.,* 2021; Ayoub *et al.,* 2019; Solomon *et al.,* 2006). It is also necessary to note that figs have long been utilized for their therapeutic properties as an anti-inflammatory, cardiovascular, respiratory, laxative, and antispasmodic medicine (Guarrera, 2005; Duke *et al.,* 2002). Many plant parts, including bark, leaves, tender shoots, fruits, seeds, and latex, have therapeutic value and are used to treat a variety of illnesses (Joseph and Justin, 2011). However, fruits have diuretic, expectorant, and mild laxative properties (Solomon *et al.,* 2006).

In addition to being used as a purgative and vermifuge, latex is frequently used to treat warts, skin ulcers, and wounds (Joseph and Justin, 2011). Furthermore, it has been demonstrated that fig latex includes a strong cytotoxic agent that inhibits the growth of numerous cancer cell lines *in vitro* (Purnamasari *et al.,* 2019; Zhang *et al.,* 2018; Yancheva *et al.,* 2005; Rubnov *et al.,* 2001). Extracts from the leaves of *F. carica* have been shown to have antidiabetic properties. The most notable drop in serum glucose levels was 8% for *F. carica* leaves fed a baseline diet (El-shobaki *et al.,* 2010). When rats with

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streptozotocin-induced diabetes were given an aqueous decoction of fig leaves, their total cholesterol level and the ratio of total cholesterol to HDL cholesterol decreased, and their hyperglycemia decreased as well (Canal *et al.,* 2000; Perez *et al.,* 1998). Stalin *et al.* (2012) recently tested the methanolic leaf extract of *F. carica's* antidiabetic properties in rats with diabetes induced by alloxan. The leaf decoction has long been used to cure liver and kidney calcifications, as well as diabetes.

F. carica leaf extract may be a useful supplement for regulating the levels of triglycerides and total cholesterol in poultry liver, according to several studies (Pucci *et al.,* 2022; Asadi *et al.,* 2006). Numerous studies have shown that antioxidants from figs can inhibit the oxidation of plasma lipoproteins and induce a marked increase in plasma antioxidant capacity for up to 4 h following ingestion (Vinson *et al.,* 2005). It is unclear what mechanism causes such an effect. *F. carica* has been the subject of numerous pharmacological investigations, the majority of which have depended on uncharacterized crude extracts. However, its wide range of traditional applications and documented biological activities highlights the significant potential for additional phytochemical research that still exists. Our current study aimed to assess the phytochemical composition, antibacterial and antioxidant activity of mature leaves of six *F.carica* types grown in the Mahdia region of Tunisia.

2. Materials and Methods

2.1 Plant material

Leaves of *Ficus carica* L. were collected during the spring of 2020 at the fully mature stage from six representative cultivars (Besbessi, Bidhi, Bither Abiadh, Hemri, Khedhri, and Soltani) located in the Mahdia region of central-eastern Tunisia. The selection of cultivars was based on their seasonal availability and optimal productivity. Botanical identification was carried out by Dr. Naceur Elayeb, a Botanist at the Higher Institute of Applied Sciences and Technology (ISSAT), University of Monastir. No Voucher Specimens were deposited in a publicly accessible Herbarium. After being cleaned and allowed to air dry for a week at room temperature, the fresh leaves were ground into a fine powder in a food processor and kept at 4°C until they could be further examined.

2.2 Phytochemical analysis

2.2.1 Sugar content

Sugars were extracted using distilled water maintained at 100°C. Reducing sugars (fructose and glucose) were quantified both prior to and following the hydrolysis of sucrose, in accordance with the method described by Miller (1959). Sucrose hydrolysis was performed using 1/ N HCl in a water bath at 67°C for 10 min, following the removal of interfering proteins by precipitation as outlined by Brunel (1949). Non-reducing sugars (sucrose) were determined by calculating the difference in reducing sugar content before and after acid hydrolysis. Results were expressed as grams of glucose per 100 g of dry weight (g glucose/100/g DW).

2.2.2 Protein content

The protein content was determined using the Kjeldahl method. One gram of powdered leaf material was digested in a Kjeldahl flask with 10 ml of concentrated sulfuric acid (H, SO_2) and a Kjeldahl catalyst tablet containing potassium sulfate (K, SO_2) and copper sulfate ($CuSO_2$) until the solution became clear. The digest was then filtered into a 250 ml volumetric flask and diluted to volume with distilled water. Steam distillation was subsequently performed after the addition of 50 ml of 45% sodium hydroxide (NaOH) to the digest, releasing ammonia, which was collected in an Erlenmeyer flask containing 25 ml of boric acid solution and 1 ml of 5% methyl red indicator. The collected distillate was immediately titrated with 0.1 N hydrochloric acid (HCl). The protein content was expressed as grams per 100 g of dry weight (g/100/g DW).

2.2.3 Lipid profile

Total lipids were extracted from the leaf samples using a chloroform–methanol mixture (2:1, v/v) supplemented with 0.01% butylated hydroxytoluene (BHT) as an antioxidant, following a modified version of the method described by Folch *et al.* (1957). Lipid methylation was carried out using a reagent mixture consisting of methanol, hexane, and sulfuric acid (75:25:1, v/v). The transmethylated lipid aliquots were then heated at 90°C for 90 min. Fatty acid methyl esters (FAMEs) were analyzed in triplicate using gas chromatography (Hewlett Packard, Palo Alto, CA) equipped with a flame ionization detector (FID) and a polar fused silica capillary column (HP-Innowax, 30 m × 0.25 mm i.d., 0.25 µm film thickness; cross-linked PEG, Carbowax 20M). The oven temperature was programmed to increase from 180°C to 250°C at a rate of 10°C per min. The injector and detector temperatures were set at 220°C and 280°C, respectively. FAMEs were identified by comparing their retention times with those of standard compounds. The results were expressed as percentages of total fatty acids.

2.2.4 Total phenols and o-diphenols content

At room temperature, 0.5 g of dried leaf powder was subjected to three successive extractions with methanol under continuous agitation for 24 h in the dark using a mechanical shaker. Following decantation and filtration, all methanolic extracts were stored at –20°C until further analysis. The determination of total phenolic content and o-diphenols was performed following a modified version of the method described by Montedoro *et al.* (1992). For total phenol quantification, 0.4 ml of each extract was mixed with 10 ml of diluted Folin-Ciocalteu reagent. After the addition of 8 ml of sodium carbonate solution (75/ g/l), the mixture was incubated for 2 h in the dark. Absorbance was then measured at 765 nm using a spectrophotometer. To quantify o-diphenols, absorbance was measured at 500 nm after a 30 min incubation. Results were expressed as milligrams of hydroxytyrosol (HYD) equivalents per 100 g of dry weight (mg HYD/100/g DW).

2.2.5 Total flavonoid content

The total flavonoid content of the methanolic leaf extracts was determined using a colorimetric assay as described by Zhishen *et al.* (1999). Briefly, 1 ml of appropriately diluted extract was mixed with 4 ml of distilled water. At time zero, 0.3 ml of 5% (w/v) sodium nitrite ($NaNO_2$) solution was added. After 5 min, 0.3 ml of 10% (w/v) aluminum chloride ($AlCl_2$) solution was introduced. Following an additional 6 min incubation, 2 ml of 1 M sodium hydroxide (NaOH) was added, and the final volume was adjusted to 10 ml with 2.4 ml of distilled water. The mixture was thoroughly homogenized, and absorbance was measured at 510 nm. Total flavonoid content was expressed as milligrams of catechin equivalents (CEQ) per 100 g of dry weight (mg CEQ/100/g DW).

2.2.6 Total anthocyanin content

The total anthocyanin content of fig leaves was determined following the method of Padmavati *et al.* (1997), with modifications by Chung *et al.* (2005). Briefly, 1 g of dried leaf powder was extracted with 25 ml of acidified methanol (methanol containing 1% HCl, v/v) at room temperature in the dark for 24 h. After extraction, the mixture was centrifuged at 1,000 × g for 15 min. The absorbance of the clear supernatant was measured at 530 and 653 nm using a UV-visible spectrophotometer. The anthocyanin content was calculated using the following equation: ($A = A_{530} - (0.24 \times A_{653}$) (Gould *et al.,* 2000).

A molecular weight (MW) of 449.2 g mol^{-1} and a molar extinction coefficient (ε) of 26,900 l mol^{-1} cm^{-1} at 530 nm were used to calculate the total anthocyanin content, which was represented as mg cyanidin-3-glucoside equivalents per 100 g dry weight (DW). The following equation was used:

$$\text{Total anthocyanins (mg/100 g DW)} = (A \times MW \times V \times 100) / (\varepsilon \times G)$$

where,

V = extract volume (ml),

ε = molar extinction coefficient (26,900),

G = sample weight (g DW),

A = adjusted absorbance, and

MW = molecular weight of cyanidin-3-glucoside (449.2).

2.2.7 Total condensed tannins content

Condensed tannins were quantified following a modified version of the method described by Sun *et al.* (1998). Briefly, 750 µl of sulfuric acid (H, SO_2) was mixed with 1.5 ml of 4% vanillin solution in methanol and 50 µl of the methanolic leaf extract. The reaction mixture was incubated in the dark for 15 min. Absorbance was measured at 500 nm, and condensed tannin content was calculated using a tannic acid standard calibration curve. Results were expressed as milligrams of tannic acid equivalents per 100 g of dry weight (mg TAE/100/g DW).

2.2.8 Pigment content

Total carotenoids were extracted following the method of Talcott and Howard (1999) with minor modifications. Briefly, 2 g of the sample was extracted using 25 ml of an acetone/ethanol mixture (1:1, v/v) containing 200 mg/l butylated hydroxytoluene (BHT) as an antioxidant. The mixture was centrifuged at 1500 × g for 15 min at 4-5°C. The supernatant was collected, and the residue was re-extracted using the same procedure until it became colorless. The combined supernatants were then adjusted to a final volume of 100 ml with the extraction solvent. Absorbance was measured at 470 nm. Total carotenoid content was calculated using the following equation and expressed as milligrams per 100 g of dry weight (mg/100 g DW):

$$\text{Total carotenoids} = Ab \times V \times 10^6 / A^{1\%} \times 100\ G$$

G : The sample weight (g),

V : The extract's total volume,

$A^{1\%}$: The extinction coefficient for a 1% carotenoid mixture at 2500, and

Ab : Absorbance at 470 nm.

Chlorophyll extraction was performed according to the method of Lichtenthaler and Buschmann (2001). Briefly, 0.25 g of leaf material was ground in a mortar and pestle with 80% (v/v) acetone. The resulting homogenate was filtered through two layers of nylon mesh and subsequently centrifuged at 15,000 × g for 5 min in sealed tubes. The supernatant was collected, and absorbance readings were taken at 663 nm and 647 nm to quantify chlorophyll a and chlorophyll b, respectively. Chlorophyll concentrations were expressed as micrograms per milliliter (µg/ml) of the extract solution.

$$Chl_a = 12.25A_{663} - 2.79A_{647}$$

$$Chl_b = 21.50A_{647} - 5.10A_{663}$$

where,

Chl_a = chlorophyll a;

Chl_b = chlorophyll b;

A_β = absorbance at λ (nm).

2.3 Antioxidant activity

2.3.1 DPPH radical scavenging activity

The antioxidant capacity of fig leaf extracts was evaluated using a slightly modified version of the DPPH free radical scavenging assay described by Kontogiorgis and Hadjipavlou-Litina (2005). Briefly, 500 µl of DPPH solution (0.1 mM in methanol) and 400 µl of Tris-HCl buffer (0.1 M, pH 7.4) were mixed with 10 µl of the sample stock solution, which was previously diluted with methanol to a final volume of 100 µl. The reaction mixture was incubated in the dark at room temperature, and absorbance was recorded at 517 nm over a 30 min period. The scavenging activity (%) was calculated using the following formula:

$$\text{Inhibition (\%)} = [(A \text{ blank} - A \text{ sample}) / A \text{ blank}] \times 100$$

A blank: the absorbance of the sample (without extract), A sample: the absorbance in the presence of the extract.

2.3.2 Reducing power

The method outlined by Oyaizu (1986) was used to evaluate the reduction capabilities of the extracts from fig leaves and barks. Each extract was combined with a 0.25 ml aliquot of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and the mixture was incubated for 20 min at 50°C. After stopping the reaction with 0.25 ml of 1% trichloroacetic acid, the liquid was centrifuged for 10 min at 3750 rpm. The absorbance was recorded at 700 nm with the addition of 0.1% $FeCl_3$ (0.125 ml), 0.5 ml of distilled water, and the supernatant (0.5 ml). The reducing powers (RP) of the tested samples rise with the absorbance values; RP is expressed in milligrams of ascorbic acid per milliliter.

2.3.3 Antibacterial activity

The antibacterial activity of the extracts was evaluated using the well diffusion method by measuring the diameter of the inhibition zones. The bacterial strains tested included *Salmonella enteritidis*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa*. A bacterial inoculum of 10^{-7} colony-forming units per milliliter (CFU/ml) was uniformly spread onto agar plates. Wells were aseptically created in the agar using a sterile Pasteur pipette, and each well was filled with 20 to 50 µl of the test extract. Positive

controls consisted of standard antibiotic discs, while negative controls used the extract solvent diluent. Plates were incubated at 37°C for 24 h, after which the diameter of the inhibition zones around each well was measured to determine antibacterial efficacy.

2.4 Statistical analysis

Data were reported as mean ± standard deviation (SD) based on three replicates, each consisting of 10 leaves per cultivar. Statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was applied to evaluate differences among groups, followed by Duncan's multiple range test to identify significant differences between means at $p<0.05$. Pearson's correlation test was conducted to assess relationships between variables.

3. Results

3.1 Sugars, proteins and pigments

F. carica leaves contain lower amounts of proteins and carbohydrates compared to the fruit (Table 1). On average, protein and total sugar concentrations were 0.95 and 0.42 g 100g dry weight (DW), respectively. Among the cultivars, Bither Abiadh exhibited the highest total sugar content (0.48 g/100 g DW), while the Bidhi cultivar showed the lowest (0.39 g/100 g DW). Reducing sugars were present only in trace amounts, averaging 0.15 g 100 g DW. The Hemri cultivar's leaves contained the highest protein concentration (1.09 g/100 g DW) and the lowest reducing sugar content (0.08 g/100 g DW), compensated by a relatively high sucrose level (0.34 g/100 g DW), which accounted for nearly 82% of its total sugar content. Moreover, chlorophyll, the green pigment responsible for capturing light energy in photosynthesis and thus essential for carbohydrate synthesis, was most abundant in the Bither Abiadh cultivar (41.72 µg/ml), which may contribute to its elevated sugar levels, as shown in Table 1.

3.2 Phenolic compounds

Phenolic compounds, including o-diphenols, total phenols, anthocyanins, total flavonoids, and condensed tannins, along with pigment contents such as carotenoids, chlorophyll a, and chlorophyll b, were quantified and are presented in Table 1. The phenolic content across selected fig leaf cultivars exhibited considerable variability. The total phenols averaged 990.87 mg hydroxytyrosol (HYD) equivalents per 100 g dry weight (DW), ranging from 819.80 to 1071.29 mg HYD/100 g DW. Flavonoid content ranged from 131.62 to 162.21 mg catechin equivalents (CEQ) per 100 g DW, with an average of 143.52 mg CEQ/100 g DW. Anthocyanin levels varied between 211.26 and 362.77 mg per 100 g DW, averaging 274.38 mg/100 g DW. Similarly, the amount of tannins ranged from 196.24 to 370.66 mg tannic acid 100 g DW, with an average of 261.46 (mg tannic acid/100 g DW).

Table 1: **Sugars, proteins, phenolic compounds and pigment contents of Tunisian *F. carica* leaves cultivars**

	Cultivars					
	Besbessi	**Bidhi**	**Bither abiadh**	**Hemri**	**Khedhri**	**Soltani**
Sugars (g/100 g DW)						
Total sugars	0.46 ± 0.03^{bc}	0.39 ± 0.03^{a}	0.48 ± 0.03^{c}	0.41 ± 0.02^{ab}	0.42 ± 0.01a^{bc}	0.40 ± 0.01^{a}
Reducing sugars	0.20 ± 0.01^{c}	0.12 ± 0.02^{b}	0.20 ± 0.01^{c}	0.08 ± 0.01^{a}	0.21 ± 0.01^{c}	0.12 ± 0.01^{b}
Sucrose	0.27 ± 0.05^{ab}	0.28 ± 0.07^{ab}	0.27 ± 0.03^{ab}	0.34 ± 0.01^{b}	0.21 ± 0.03^{a}	0.27 ± 0.01^{ab}
Proteins (g/100 g DW)	0.81 ± 0.02^{a}	1.03 ± 0.01^{b}	1.04 ± 0.06^{b}	1.09 ± 0.01^{b}	0.73 ± 0.01^{a}	1.05 ± 0.03^{b}
Pigments						
Chlorophyll a (µg ml^{-1})	26.36 ± 0.16^{b}	26.03 ± 2.31^{b}	26.48 ± 0.34^{b}	27.42 ± 1.71^{b}	24.69 ± 2.18^{ab}	22.84 ± 0.23^{a}
Chlorophyll b (µg ml^{-1})	14.60 ± 0.69^{d}	11.55 ± 0.65^{c}	15.24 ± 1.48^{d}	11.74 ± 0.26^{c}	7.39 ± 0.99^{a}	9.87 ± 0.46^{b}
Total chlorophylls (µg ml-1)	40.77 ± 0.72^{bc}	37.58 ± 2.96^{b}	41.72 ± 1.81^{c}	39.16 ± 1.65^{bc}	32.09 ± 1.70^{a}	32.72 ± 0.63^{a}
Total carotenoids (mg/100 g)	126.6 ± 1.58^{b}	126.0 ± 1.95^{b}	119.3 ± 10.80^{ab}	127.1 ± 1.00^{b}	115.5 ± 3.47^{a}	118.8 ± 2.30^{ab}
Phenolic compounds (mg/100 g DW)						
Total phenols	819.8 ± 18.01^{a}	975.8 ± 20.50^{b}	1060.9 ± 67.52^{c}	1071.2 ± 11.01^{c}	1040.9 ± 0.39^{c}	976.40 ± 4.12^{b}
o-diphenols	92.66 ± 8.97^{ab}	80.85 ± 0.98^{ab}	92.83 ± 10.97^{ab}	96.76 ± 1.03^{b}	82.92 ± 8.54^{ab}	79.77 ± 10.65^{a}
Flavonoids	121.5 ± 29.11^{a}	149.2 ± 9.31^{ab}	142.84 ± 11.44^{ab}	162.21 ± 14.62^{b}	136.85 ± 18.65^{ab}	131.61 ± 12.36^{ab}
Total anthocyanins	362.7 ± 9.97^{c}	283.5 ± 10.10^{b}	297.20 ± 18.83^{b}	265.07 ± 24.48^{ab}	211.26 ± 38.25^{a}	244.48 ± 19.23^{ab}
Tannins	278.1 ± 3.13^{b}	221.9 ± 11.00^{ab}	196.24 ± 5.09^{a}	370.66 ± 36.28^{c}	251.14 ± 32.89^{ab}	250.68 ± 19.12^{ab}

Means ± standard deviation are used to express values (n = 3). At the $p<0.05$ level, means with distinct letters were substantially different.

3.3 Fatty acids

Table 2 summarises the fatty acid profiles of the selected fig leaf cultivars analysed in this study. Saturated fatty acids (SFA) made up approximately 50% of the total fatty acid content, followed by polyunsaturated fatty acids (PUFA) at 37%, and monounsaturated fatty acids (MUFA) at 13%. The Bidhi cultivar exhibited the highest SFA content at 58.41%, while the Hemri cultivar had the lowest at 41.71%. The predominant SFAs identified were palmitic acid (C16:0), stearic acid (C18:0), and arachidic acid (C20:0), accounting for

22.88%, 9.75%, and 7.91% of total fatty acids, respectively. Among MUFAs, oleic acid (C18:1) was the most abundant, averaging 9.95% of total fatty acids, with the lowest proportion observed in the Khedhri cultivar (6.67%) and the highest in the Bidhi cultivar (16.43%). For PUFAs, linolenic acid (C18:3) and linoleic acid (C18:2) were predominant, contributing 17.53% and 19.13%, respectively, to the total fatty acid profile. Remarkably, linolenic acid reached a high concentration of 27.78% in the Hemri cultivar.

Table 2: Fatty acid composition (%) evaluated by GC in leaves of Tunisian *F.carica* cultivars

Fatty acids (%)	Cultivars					
	Besbessi	Bidhi	Bither abiadh	Hemri	Khedhri	Soltani
C12:0	0.95 ± 0.02^{a}	1.56 ± 0.14^{a}	1.08 ± 0.07 ^{a}	1.00 ± 0.30^{a}	1.17 ± 0.04^{a}	1.81 ± 0.98^{a}
C14:0	0.82 ± 0.07^{ab}	0.96 ± 0.03^{ab}	0.40 ± 0.44^{a}	1.07 ± 0.30^{b}	0.87 ± 0.00^{ab}	1.37 ± 0.17^{b}
C16:0	22.5 ± 0.12^{ab}	31.7 ± 0.14^{b}	25.7 ± 3.35^{ab}	15.0 ± 10.5^{a}	25.5 ± 3.77^{ab}	16.6 ± 0.79^{a}
C17:0	2.10 ± 1.98^{a}	4.72 ± 0.07^{a}	3.39 ± 0.02^{a}	4.65 ± 1.73^{a}	5.12 ± 0.28^{a}	8.88 ± 2.62^{b}
C18:0	15.7 ± 0.50^{c}	9.69 ± 0.21^{ab}	7.90 ± 1.81^{ab}	10.2 ± 3.60^{b}	9.24 ± 1.27^{ab}	5.63 ± 0.73^{a}
C20:0	6.21 ± 1.29^{a}	8.80 ± 0.15^{a}	6.28 ± 1.48^{a}	7.68 ± 3.23^{a}	6.80 ± 0.99^{a}	11.6 ± 4.17^{a}
C22:0	0.35 ± 0.02^{a}	0.60 ± 0.11^{a}	0.29 ± 0.11^{a}	068 ± 0.33^{a}	0.38 ± 0.19^{a}	0.60 ± 0.37^{a}
C23:0	0.19 ± 0.04^{ab}	0.15 ± 0.04^{ab}	0.15 ± 0.01^{ab}	0.10 ± 0.02^{a}	0.23 ± 0.02^{b}	0.13 ± 0.04^{a}
C24:0	1.51 ± 0.17^{abc}	0.13 ± 0.02^{a}	1.85 ± 0.05^{abc}	1.25 ± 0.14^{ab}	2.67 ± 1.11^{bc}	3.29 ± 1.35^{c}
ΣSFA	**50.48 ± 0.16^{abc}**	**58.41 ± 0.96^{c}**	**47.08 ± 2.87^{ab}**	**41.71 ± 1.20^{a}**	**52.10 ± 2.39^{bc}**	**50.11 ± 8.20^{abc}**
C12:1	0.09 ± 0.05^{a}	0.26 ± 0.02^{b}	0.08 ± 0.07^{a}	0.14 ± 0.06^{ab}	0.08 ± 0.06^{a}	0.22 ± 0.09^{ab}
C14:1	0.09 ± 0.05^{a}	0.15 ± 0.03^{a}	0.95 ± 0.31^{bc}	0.58 ± 0.34^{ab}	1.34 ± 0.00^{c}	0.44 ± 0.15^{ab}
C16:1	0.80 ± 0.31^{b}	0.24 ± 0.05^{a}	0.15 ± 0.08^{a}	0.14 ± 0.05 ^{a}	0.14 ± 0.06^{a}	0.05 ± 0.01^{a}
C17:1	1.14 ± 0.19^{a}	0.86 ± 0.02^{a}	0.76 ± 0.38 ^{a}	0.66 ± 0.04 ^{a}	1.23 ± 0.14^{a}	0.93 ± 0.67^{a}
C18:1	10.3 ± 0.82^{b}	16.4 ± 0.41^{c}	8.68 ± 2.01^{ab}	8.75 ± 0.42 ^{ab}	6.67 ± 0.10^{a}	8.81 ± 2.29^{ab}
C20:1	1.48 ± 0.74^{a}	1.35 ± 0.11^{a}	0.90 ± 0.12^{a}	1.42 ± 0.53^{a}	0.83 ± 0.19^{a}	0.59 ± 0.13^{a}
ΣMUFA	**14.00 ± 1.16^{b}**	**19.31 ± 0.46^{c}**	**11.52 ± 2.19^{ab}**	**11.69 ± 0.27^{ab}**	**10.31 ± 0.32^{a}**	**11.05 ± 2.00^{ab}**
C18:2	14.83 ± 0.00 ^{a}	21.87 ± 0.09 ^{a}	16.74 ± 0.88 ^{a}	18.01 ± 6.20 ^{a}	12.38 ± 2.36 ^{a}	21.39 ± 9.57 ^{a}
C18:3	21.12 ± 0.10 ^{bc}	0.39 ± 0.06 ^{a}	23.82 ± 5.84 ^{bc}	27.78 ± 6.90 ^{c}	24.97 ± 4.42 ^{bc}	16.74 ± 1.43 ^{b}
C20:2	1.08 ± 0.04^{d}	0.42 ± 0.07^{ab}	0.74 ± 0.14^{bc}	1.04 ± 0.24^{cd}	0.28 ± 0.13^{a}	1.15 ± 0.00^{d}
ΣPUFA	**37.03 ± 0.14^{b}**	**22.69 ± 0.24^{a}**	**41.30 ± 5.09^{b}**	**46.84 ± 0.95^{b}**	**37.63 ± 1.93^{b}**	**39.29 ± 11.00^{b}**

Means ± standard deviation are used to express values (n = 3). At the $p<0.05$ level, means with distinct letters were substantially different.

3.4 Antioxidant activity

The antioxidant activity of fig leaf extracts was evaluated using two complementary assays: the DPPH free radical scavenging test and the reducing power (RP) assay, as presented in Figure 1. The DPPH assay revealed a notably high antioxidant capacity across all cultivars, with an average free radical scavenging activity of 84.26%. This strong activity is likely attributed to the rich phenolic composition of the fig leaves, including high levels of total phenols and flavonoids. The RP assay, which is based on the reduction of the $Fe^{3}z$ ferricyanide complex to its ferrous form, evident by a colour change from yellow to green or blue, provides further insight into the antioxidant potential of the extracts. The intensity of this colour shift, measured in milligrams of ascorbic acid equivalents per milliliter (mg AAE/ml), correlates directly with the sample's reducing capacity (Ferreira *et al.,* 2007). As shown in Figure 1, the Hemri cultivar demonstrated the highest reducing power, which may be linked to its elevated levels of total phenols and flavonoids, surpassing those observed in the Besbessi cultivar.

3.5 Antibacterial activity

Methanolic extracts of *F. carica* leaves did not exhibit any detectable antibacterial activity, as no inhibition zones were observed against the tested bacterial strains under the applied experimental conditions.

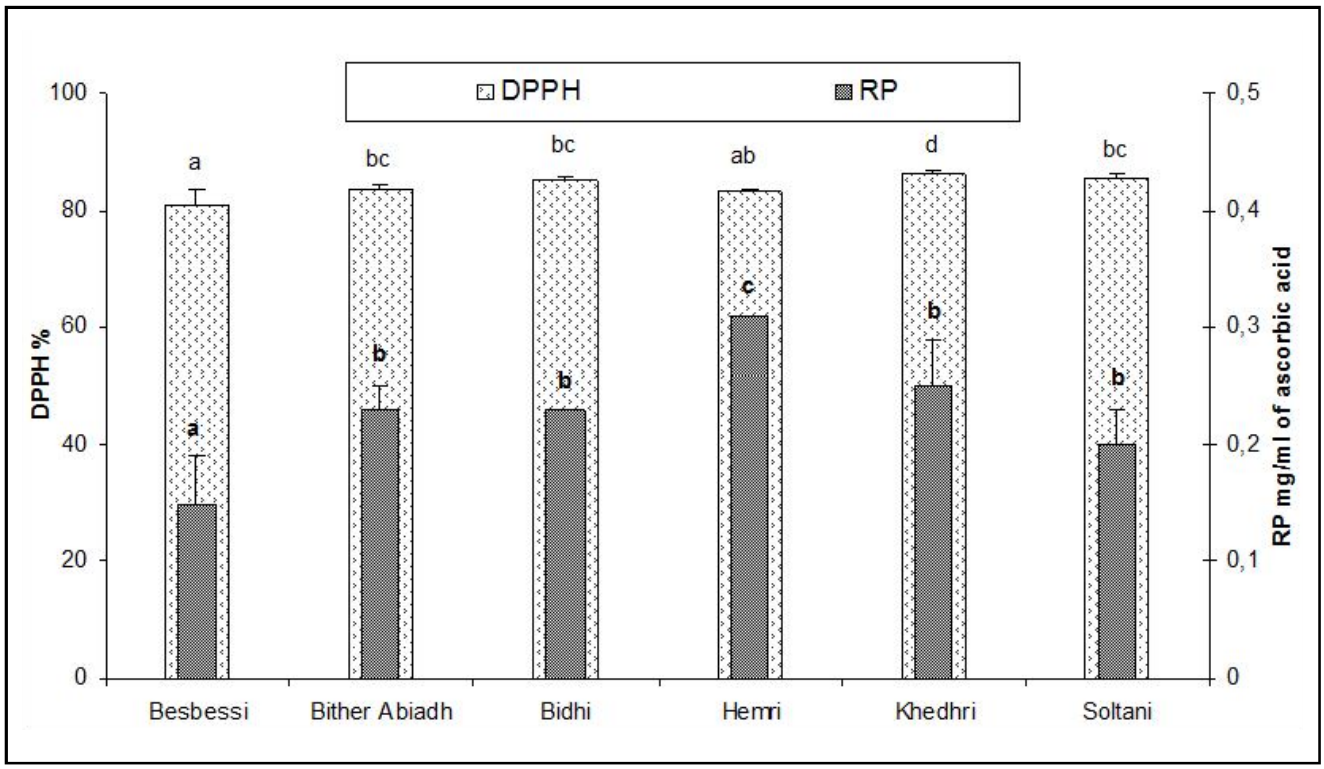

Figure 1: Antioxidant activity of six cultivars of fig leaves evaluated with DPPH and RP tests. The mean ± SD of three replicates of 10 leaves each per cultivar is used to report the data. Cultivar differences that are significant at $p<0.05$ according to Duncan's test are indicated by the letters a, b, c, and d.

4. Discussion

F. carica leaves exhibited a lower concentration of sugars and proteins in comparison to the fruit. Interestingly, all the cultivars examined demonstrated a high proportion of sucrose, accounting for an average of 65% of the total sugar content, while the levels of reducing sugars (fructose and glucose) remained relatively low. This observation aligns with the findings of Vemmos *et al.* (2013), who reported that fructose, glucose, and sucrose are the primary soluble sugars in both fig fruits and leaves. Specifically, their study highlighted that sucrose was significantly more abundant in fig leaves across all cultivars compared to other sugars. This pattern is in agreement with the general understanding that sucrose serves as the major carbohydrate translocated from source tissues (leaves) to sink tissues (*e.g.,* fruits) in most fruit trees (Kozlowski *et al.,* 1992).

Phenolic compounds are important secondary metabolites that, in addition to sugars and organic acids, contribute to the sensory profile of fruits by influencing aroma and, to a lesser extent, sweet, bitter, or astringent tastes (Tomas-Barberan and Espín, 2001). Beyond their role in flavour, phenolics are increasingly recognized for their health-promoting properties, which has drawn considerable interest from consumers and researchers alike. Fig (*F. carica*) leaves, in particular, have been identified as a rich and economical source of bioactive phenolics. Oliveira *et al.* (2009) reported that the phenolic content in fig leaves was substantially higher than in other parts of the plant, with the aqueous lyophilized leaf extracts showing the highest concentrations, followed by peel and then pulp. This distribution pattern is consistent with other studies suggesting that leaves typically accumulate more phenolic compounds than fruits (Ammar *et al.,* 2015), likely due to their role in protecting photosynthetic tissues from ultraviolet (UV) radiation. Phenolics, especially flavonoids, function as natural UV filters and help safeguard chloroplasts and other organelles from oxidative damage (Treutter, 2006; Macheix *et al.,* 1990).

Our findings further support these observations, with the Hemri cultivar distinguished by its red fruit skin exhibiting the highest concentrations of total phenols, o-diphenols, flavonoids, condensed tannins, chlorophyll a, and carotenoids. In contrast, the Besbessi cultivar, which bears green-skinned fruits, had the lowest levels of total phenols (819.80 mg HYD/100 g DW) and flavonoids (121.53 mg CEQ/100 g DW). These trends are in agreement with prior reports indicating that darker-colored fruits, particularly those with red, blue, or purple pigmentation, tend to possess higher antioxidant capacities (Liu *et al.,* 2002; Wu *et al.,* 2006; Solomon *et al.,* 2006; Celik *et al.,* 2008). Notably, Solomon *et al.* (2006) highlighted that fig cultivars such as Mission and Chechick, which exhibit dark purple skin, contained higher phenolic contents compared to their lighter-skinned counterparts.

The fatty acid profile of *F. carica* leaves revealed that saturated fatty acids (SFA) constituted the majority of the total fatty acid composition, accounting for more than 50%, followed by polyunsaturated fatty acids (PUFA) at 37% and monounsaturated fatty acids (MUFA) at 13%. Among PUFAs, linoleic acid (C18:2) and linolenic acid (C18:3) were the predominant constituents, contributing 17.53% and 19.13%, respectively, to the total fatty acid content. These findings align with those of Marrelli *et al.* (2014), who also reported linolenic acid, stearic acid, and linoleic acid as the principal fatty acids in *F. carica* leaf extracts.

Antioxidant activity was evaluated using both ferric reducing power (RP) and DPPH radical scavenging assays. The results indicated substantial antioxidant capacity, likely attributed to the high levels of phenolic compounds in the extracts. Statistical analysis revealed a strong positive correlation between total phenolic content and both DPPH scavenging activity (r = 0.688) and reducing power (r = 0.791), significant at $p<0.01$. These observations reinforce the hypothesis that phenolic compounds significantly contribute to the antioxidant potential of fig leaves. This is consistent with earlier studies that demonstrated a similar association between total phenolic content and antioxidant activity in figs (Solomon *et al.,* 2006; Veberic *et al.,* 2008). Moreover, flavonoid content showed a moderate positive correlation with reducing power (r = 0.523, $p<0.05$), while total anthocyanin content exhibited a significant inverse correlation with DPPH activity (r = -0.848, $p<0.01$), suggesting a complex interaction among different phenolic classes in modulating antioxidant effects.

The methanolic extracts of *F. carica* leaves did not exhibit antibacterial activity against the tested bacterial strains, as no inhibition zones were observed. This finding is consistent with the results of Oliveira *et al.* (2009), who also reported a lack of antibacterial effects from methanolic leaf extracts against similar bacterial species. In contrast, Jeong *et al.* (2009) demonstrated that methanolic extracts of *F. carica* leaves exhibited strong antibacterial activity against several oral pathogens, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Streptococcus gordonii*, and *Streptococcus anginosus*.

5. Conclusion

This study highlights the significant biochemical diversity among *F. carica* leaf cultivars, particularly in terms of phenolic content, antioxidant capacity, and fatty acid composition. The Hemri cultivar, distinguished by its high levels of phenolic compounds and strong antioxidant activity, emerges as a promising source of natural antioxidants. Additionally, the fatty acid profile, predominantly composed of saturated and polyunsaturated fatty acids, emphasizes the nutritional value of fig leaves. Although, no antibacterial activity was observed under the tested conditions, the rich phytochemical composition supports the potential use of fig leaves as a functional dietary supplement. These findings warrant further exploration into the valorization of fig leaves for applications in functional foods, nutraceuticals, and pharmaceutical formulations.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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