ARTICLE

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Assessment of flavonoid-rich extracts from dark peels of *Ficus carica* L. fruits for cosmeceutical and antimicrobial applications

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Author contributions

Leila Meziant conceived the study, performed the analysis and wrote the original draft. Mostapha Bachir-Bey supervised and corrected the manuscript. Malika Boutiche was involved in the investigation and resources. Lynda Gali and Assia Ikhlef implicated in the investigation and performed some analyses. Hayette Louaileche was responsible for conceptualization and involved in resources.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

F. carica, *Ficus carica*; SPF, sun protection factor; FPE, fig peel extracts; UV, ultraviolet; ATCC, American Type Culture Collection; CFU, colony-forming unit; MIC, minimal inhibitory concentration; MED, minimal erythemal dose; DM, dry matter; LC_{50}, lethal concentration of 50%; IC_{50}, half-maximal inhibitory concentration.

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Abstract

Background: Exploring natural sources to identify new active ingredients for cosmeceutical and pharmaceutical applications is of interest to many researchers. **Methods:** Flavonoid-rich extracts from dark *Ficus carica* peels were investigated in the present study. Extracts were prepared from the peels of four dark cultivars (Bakor Noir, Bouankik, Azenjer and Tazegaght) using acidified methanol. The antimicrobial activity of the extracts was evaluated by the agar well diffusion assay followed by the minimal inhibitory concentration determination using the microdilution method against Gram-positive and Gram-negative pathogenic bacteria and two fungi. Cosmeceutical properties were evaluated by measuring antioxidant activity, ultraviolet absorption characteristics and sun protection factor values. The antioxidant activity of the extracts was measured using a β-carotene-linoleic acid system. The toxicity of the extracts was assessed using a brine shrimp lethality assay. **Results:** The extracts contained high amounts of phenolic compounds (3.85–8.63 g/100 g), mainly flavonoids (up to 5 g/100 g). Multiple antibacterial activities were recorded against Gram-positive and Gram-negative bacteria, with the best actions against *Bacillus subtilis* (minimal inhibitory concentration = 156.25 μg/mL), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (minimal inhibitory concentration = 312.5 μg/mL), but no antifungal activity was recorded. *Azenjer* extract showed high inhibition of lipid oxidation evaluated by the β-carotene bleaching assay with a half-maximal inhibitory concentration of 88.84 μg/mL. All extracts showed absorption peaks in the ultraviolet range and *Azenjer* and *Tazegaght* extracts at 25 mg/mL presented sun protection factor values > 15. The brine shrimp toxicity assay results revealed lethal concentration of 50% values greater than 2,000 μg/mL, indicating the safety of the extracts. **Conclusion:** Therefore, *Ficus carica* peel extracts could be considered original ingredients with potential applications in the formulation of antibacterial drugs and skincare products.

Keywords: *Ficus carica*; flavonoids; antioxidant activity; antimicrobial activity; sun protection factor; brine shrimp toxicity

Highlights

Flavonoid-rich extracts prepared from four dark cultivars of *Ficus carica* fruits showed a strong antioxidant capacity comparable to ascorbic acid and an inhibitory effect against both Gram (+) and Gram (−) pathogenic bacteria, especially *Bacillus sublitis* and *Staphylococcus aureus*. Extracts at 25 mg/mL are potent ultraviolet-photoprotective agents with sun protection factor higher than 15 without showing any toxic effect against brine shrimps, indicating that dark *Ficus carica* fruit peel extracts are original sources of pharmaceutical and cosmeceutical ingredients.

Medical history of objective

The medicinal and cosmetic use of *Ficus carica* fruits begins since antiquity. For more than 5,000 years ago, ancient Egyptians already used *Ficus carica* fruits to make remedies and beverages and ancient Greek physicians used figs to make poultices. The health benefit of *Ficus carica* fruits was also known in ancient Rome as testified by the writings of Pliny the Elder (23–79 C.E.), author of the encyclopedia *The Natural History*, who said that figs are restorative, increase the strength of young people, maintain the elderly in better health and make them look younger with fewer wrinkles. Roman people used figs in mixtures with other ingredients (bananas, oat flour and rose water) to make facial creams. Today, scientific research provided strong evidence of the medicinal properties of *Ficus carica* fruit extracts including the anti-diabetic, anti-proliferative, hepato-protective, cardiovascular protective, anti-inflammatory, antimicrobial, hypo-lipidemic activities among others. Numerous commercialized cosmetics and personal care products contain fig extracts to confer an exotic fragrance and as an active ingredient (moisturizing, whitening, anti-aging, exfoliating, etc.). It is believed that most pharmacological aspects of *Ficus carica* extracts are tightly related to their phyto-constituents such as phenolic compounds, flavonoids and terpenoids, given the low vitamin C and E contents of figs. Extracts with high flavonoid and anthocyanin contents showed high antioxidant and biological properties through multiple mechanisms of action (free radical scavenging, reduction of oxidized molecules, binding metal ions, photon absorption, etc.) that involves mainly the hydroxyl groups, the double binds and aromatic rings. Thereby, the extracts of *Ficus carica* fruits with high flavonoid contents are suggested as natural ingredients to use in pharmacological and cosmeceutical formulations.

Background

Natural product screening is an emergent approach developed in the last century and is based on the phytochemical and pharmacological evaluation of plant-based extracts, leading to drug discovery [1]. In addition, the use of natural sources in the development and formulation of skin products as an alternative to conventional drugs and synthetic products has increased interest in research and industrial applications of medicinal plants [2].

Ficus carica (*F. carica*) is one of the oldest medicinal plants used by humans to treat internal and external ailments. In different traditional medicines globally where *F. carica* plants are found, the preparation from different parts varies in complexity, from a simple splitting of the fruit for application as a poultice to preparing lye from the ashes of different parts of the plant [3]. All *F. carica* parts, including fruits, bark, leaves, twigs, young shoots and latex, have been used as remedies for tumors and diseases associated with inflammation [3]. The fig fruits are traditionally used for their antipyretic, anti-inflammatory, lithotriptic, emollient, and laxative characteristics in the treatment of leprosy, paralysis, liver diseases, chest pain and piles [1]. Fresh figs have also been reported to be beneficial for anemia, hepatic problems, coughs, and bronchial problems [4]. The pulp was used to relieve pain, gingival abscesses and mouth ulcers; dried fig paste was used to treat burns, eczema, and abdominal cramps; and dried fig decoction was used in the treatment of measles and smallpox [5]. Diabetic patients in Spain and southwestern Europe drink decoctions of *F. carica* leaves to lower blood sugar [6]. In Asia, poultices prepared with *F. carica* leaves were also used to treat dermatitis and tumors, and when mixed with honey or vinegar, preparations were used to treat moist ulcers with viscous exudates and running ulcers [3]. In South America, *F. carica* latex was applied to warts and verruca's as an external treatment [7]. In the Middle East, latex combined with egg yolk was used in the treatment of ulcers and an ointment of latex and fenugreek flour was used to cure gout [3].

In recent years, with the emergence of new issues related to modern lifestyles and industrialization (stress, fast food, pollution, radiation, etc.), research on *F. carica* has been directed to the investigation of multiple medicinal and pharmacological aspects as novel solutions to current health disorders (cancer, diabetes, neurodegenerative, cardiovascular, and inflammatory diseases). The main *F. carica* activities described in the literature to date include anti-diabetic, anticancer, anti-mutagenic, anti-angiogenesis, anti-proliferative, hepatoprotective, cardiovascular protective, anti-inflammatory, immunomodulatory, hypolipidemic, nephroprotective, diuretic, erythropoietic, anti-convulsant, anti-constipation, anti-pyretic, anthelmintic, wound healing, antiviral, antibacterial and antifungal activities [8–39]. In addition, many researchers are interested in the antioxidant activity of *F. carica* extracts by investigating different mechanisms, including free radical scavenging, reducing power, lipid peroxidation inhibition, metal chelating activities and even cell-based antioxidant capacity [15, 37, 40–51].

F. carica contains numerous phytoconstituents, including phytosterols (β-sitosterol, lupeol, and betulol), amino acids (leucine, tryptophan, lysine, serine and cysteine), organic acids (oxalic, citric, malic and fumaric acids), fatty acids (myristic, palmitic, oleic and linoleic acids), phenolic compounds (3-caffeoylquinic acid, ferulic acid and psoralene), flavonoids (rutin, luteolin, quercetin and cyanidin-3-rhamnoglucoside), volatile compounds (benzaldehyde, phenylethyl alcohol, linalool and caryophyllene) and a few compounds of other secondary metabolite classes found mainly in latex, leaves, fruits and roots [52]. It is believed that secondary metabolites produced by plant cells are responsible for the medicinal properties of plant extracts and have been extensively screened for multiple biological and pharmaceutical properties. With more than 5,000 plant-derived compounds, flavonoids and their glycosides are among the most important classes of secondary plant metabolites [53]. Interest in flavonoids has been stimulated by the potential health benefits arising from their high antioxidant capacity both in vitro and in vivo, conferring protective effects against cardiovascular diseases, cancers and other age-related diseases, in addition to some pathological disorders, such as gastric and duodenal ulcers, allergies, vascular fragility and viral and bacterial infections [54].

Cosmeceuticals are a combination of cosmetic and pharmaceutical products that are directly applied to the skin and can alter skin conditions [55]. Cosmeceuticals in skincare science must contain active ingredients that enhance the skin's surface and deeper layers by reducing hyperpigmentation, dilated pores, scars and wrinkles and slow aging caused principally by exposure to ultraviolet (UV) radiation, which initiates a complex chain reaction and generates harmful reactive oxygen species. These free radicals attack cellular compounds (proteins, lipids, nucleic acids, etc.), causing photodamage, inflammation and visible tissue aging [56]. Moisturizers, skin-repairing agents and sunscreen products have been the main cosmeceuticals developed in recent years. Multiple active ingredients of natural or synthetic sources, such as retinoids, carotenoids, ascorbic acid, lipoid acid, hyaluronic acid, kojic acid, allantoin, silicone, coenzyme Q10, tocopherols, curcuminoids, quercetin, resveratrol, pycnogenol, phytomelanin, phytoceramide, soy isoflavones, honey, aloe vera gels, algae extract, green tea extract, chamomile extract and other plant extracts, have already been used in commercial cosmeceutical products and stem cell-based products are now available [56, 57].

Phenolic compounds as functional ingredients have attracted significant attention for cosmeceutical and green skincare product development, particularly in sunscreen formulations, to enhance the photoprotection provided by UV filters [55]. Flavonoids are strong antioxidants that show multiple biological activities; some of them, such as rutin, also stabilize emulsions, which is an important parameter to consider in cosmeceutical products [55].

Infectious diseases caused by bacterial and fungal microorganisms affect the health of millions of people worldwide because of the global emergence of multi-drug resistant strains, which increasingly limit the effectiveness of current drugs and significantly cause treatment failure [58]. This has led to the search for new antibiotic agents from different sources and plant metabolites remain the largest and most interesting area of investigation.

Since most bioactive plant extracts are toxic at high concentrations, it is necessary to evaluate their toxicity before use. Brine shrimp (*Artemia salina*) is considered a convenient tool for pharmacological activities of plant extracts [59]. It is a simple zoological organism that has been used in many studies and has proven to be a useful tool for screening various chemical compounds in plant extracts [60]. Due to ethical issues in toxicological tests, substituting animals with alternative models is very important. The toxicity results of the brine shrimp lethality bioassay presented good correlations with toxicity results obtained from mice and rats [61, 62].

The present study aimed to contribute to the knowledge of the pharmacological properties of *F. carica* extracts by studying an original aspect related to the potential use of flavonoid-rich extracts obtained from dark peels using an optimized methodology as new cosmeceutical ingredients, taking into consideration the scarcity of research works that treat these properties on peel extracts, especially from fig fruits. The extracts were tested by assessing important aspects related to the antioxidant activity and photoprotective effects by measuring the absorption properties in the UV range and determining the sun protection factor (SPF). In an effort to search for new extracts to be used to counteract the antibiotic resistance of some human pathogenic bacterial and fungal strains, flavonoid-rich extracts from dark fig peels were evaluated against Gram-positive and Gram-negative strains and two fungi. As bioactive extracts could present toxicity, limiting their further exploitation, the toxicity of the extracts was also evaluated using the brine shrimp model.

Methods

Preparation of samples

The experimental steps, from the preparation of the samples to the recovery of the final extract, are presented in Figure 1. The present study was conducted on *F. carica* fruits of four dark cultivars, *Bakor Noir* from the region of Constantine (East Algeria); and *Bouankik*, *Azenjer* and *Tazegaght* from the region of Bejaia (North Algeria). *Bakor Noir* cultivar belongs to the common type, *Bouankik* cultivar belongs to the San Pedro type (fruits of the second crop were used) and *Azenjer* and *Tazegaght* cultivars belong to the Smyrna type. A detailed description of the four fig cultivars is reported in our previous study [47]. The fruits of each one of the four samples were peeled and the skins were lyophilized and crushed into powders kept at 4 °C in opaque containers.

Extraction process

Fig peel extracts (FPE) were obtained following the protocol described by Meziant et al. [63]. The solvent system used for the extraction was methanol, water, and 5% citric acid (72:18:10, v/v/v). Extraction was carried out at a ratio of 1:100 (solid:solvent) for 150 min at ambient temperature (25 °C). Extracts were recovered after cold centrifugation (4 °C) at 8,500 rpm for 15 min and pellets were re-extracted under the same conditions. The first and second extracts of each sample were combined to form the crude extract. An aliquot of each extract was used for phytochemical determination (total phenolics, total flavonoids, flavonols and anthocyanins) and the remaining extracts were vacuum-dried (to evaporate methanol) and freeze-dried (to eliminate humidity). The powdered extracts were reconstituted in double-distilled water at a concentration adequate for each analysis.

Phytochemical contents

The total phenolic content was determined using the Folin-Ciocalteu method. The protocol described by Velioglu et al. was followed [64]. A volume of 100 µL of FPE was mixed with 750 µL of Folin-Ciocalteu and after 5 mins, 400 µL of 10% sodium carbonate was added. After 90 min of incubation in thedark, absorbance was measured at 720 nm. The results are expressed as gallic acid equivalents (GAE) (mg GAE/100 g dry matter (DM)).

The total flavonoid content was determined following the protocol of Zhishen et al. [65]. FPE (500 µL) was mixed with an equal volume of distilled water and 150 µL sodium nitrite (5% $NaNO_2$). After 5 min, 150 µL of aluminum trichloride (10% $AlCl_3$) was added to the mixture. After 6 min of incubation, 2 mL sodium hydroxide (1 N NaOH) was added. The volume of the mixture was adjusted to 5 mL using distilled water. The mixture was vortexed and incubated for 15 min, after which absorbance was measured at 510 nm. The results are expressed as quercetin equivalents (QE) (mg QE/100 g DM).

Flavonol content was determined using the method described by

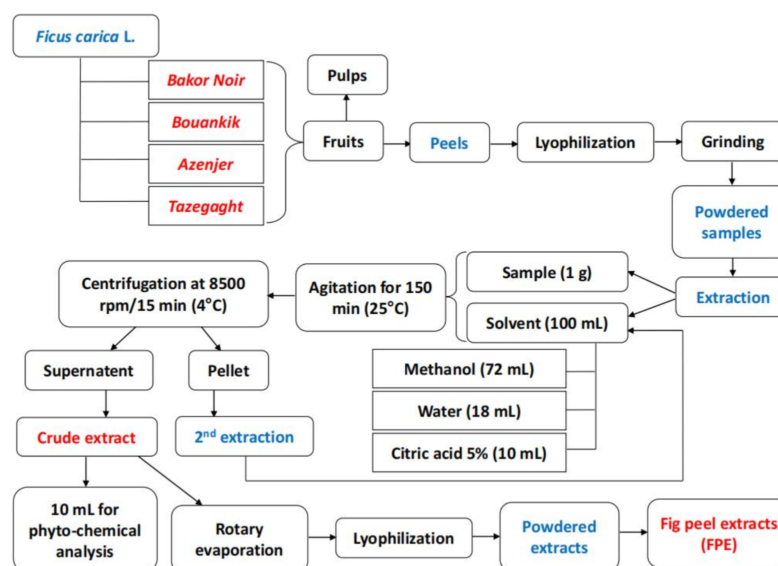

Figure 1 Scheme of the principal steps of the experimental work (samples preparation and extraction process)

Kumaran and Karunakaran [66]. The protocol consisted of mixing 500 μL of FPE with 500 μL of $AlCl_3$ (2%) and 1.5 mL of sodium acetate (5%). After 150 min of incubation, absorbance was measured at 440 nm. The results are expressed as rutin equivalents (RE) (mg RE/100 g DM).

Anthocyanin content (A_t) was determined according to Giusti and Wrolstad [67]. Extracts (two volumes for each FPE) were diluted to a final volume of 3 mL with potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5) in separate tubes. After 15 min, the absorbance of each tube was measured at 520 nm and 700 nm. The final FPE absorbance (A) was calculated as follows:

$$A = \left(A_{520} - A_{700}\right)_{\text{pH1.0}} - \left(A_{520} - A_{700}\right)_{\text{pH4.5}}$$

At were calculated according to the following equation:

$$A_t\left(\mu\text{g/mL}\right) = \frac{A \times \text{MW} \times \text{DF}}{\varepsilon \times \text{L}}$$

Where MW is the molecular weight of cyanidin-3-rutinoside (595.2 g/mol), DF is the dilution factor, and ε is the molar absorptivity of the reference (28,840 L/cm/mol), and L is the path length (1 cm).

The results were expressed as mg cyanidin-3-rutinoside equivalent (CRE) (mg CRE/100 g DM).

Determination of antimicrobial activity

Microbial strains. Three Gram-positive bacterial strains, *Bacillus subtilis* (American Type Culture Collection (ATCC) 6633), *Staphylococcus aureus* (ATCC 25923) and *Listeria monocytogenes* (ATCC 15313); three Gram-negative bacterial strains, *Salmonella enteritidis* (ATCC 13076), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853); and two fungi, *Candida albicans* (ATCC 10231) and *Aspergillus brasiliensis* (ATCC 16404), were tested in the present study. The reference pathogenic strains (ATCC) were provided by the Laboratory of Microorganisms and Bioprocesses at the Biotechnology Research Center (Constantine, Algeria). The strains were prepared in sterile water to obtain suspensions of 10^7 colony-forming unit (CFU)/mL for bacteria and 10^6 CFU/mL for fungi.

Agar diffusion assay. An agar well diffusion assay was used to investigate the antimicrobial effect of FPE, following the protocol of Duman et al. [68]. Aliquots of 15 mL of Muller-Hinton melted agar (for bacteria) or Sabouraud melted agar (for fungi) were poured into Petri plates (90 mm diameter) and left to cool. The broth was inoculated with overnight microbial suspensions to obtain a final concentration of 10^6 CFU/mL for bacteria and 10^5 CFU/mL for fungi. Approximately 5 mL of inoculated broth was poured onto the surface of the prepared Petri plates. Wells (7 mm diameter) were cut in the agar (1 well per plate), then 100 μL of FPE (20 mg/mL) or citric acid (9.5 mg/mL) was placed in each well and left to diffuse at room temperature for 30 min, then at 4 °C for two hours. The plates were incubated at 37 °C for 24 h for bacteria and at 25 °C for 48 h for fungi. Each extract was tested in triplicate. After incubation, the diameter of the inhibition zones was measured and the results were expressed in millimeters (mm). Cefazolin (5 mg/mL) was used as a positive control for the tested bacteria.

Micro-dilution assay. The minimal inhibitory concentration (MIC) of the extracts was determined using a micro-dilution assay in 96-well microplates against the bacterial strains that showed inhibition zones on agar. Muller-Hinton broth was used in the preparation of the FPE dilutions and as a culture medium for the bacterial strains. Inocula were prepared at a final concentration of 10^6 CFU/mL. In each well, 50 μL of FPE (diluted concentrations between 5 mg/mL and 9.75 μg/mL), citric acid (between 2.38 mg/mL and 4.64 μg/mL) or cefazolin (between 1.25 mg/mL and 2.44 μg/mL) was added to 50 μL of the inoculum. Each test was performed in duplicate. Microplates are incubated at 37 °C for 24 h. Sterility (without inoculum) and growth (without extract) controls were added to each microplate. MIC was defined as the lowest concentration of the extract that completely inhibited bacterial growth, as detected by the unaided eye and expressed as μg/mL.

Antioxidant activity. The antioxidant activity of FPE was evaluated using a β-carotene/linoleic acid system. Linoleic acid in aqueous emulsions is auto-oxidized and forms free radicals. These compounds are trapped by β-carotene molecules, causing discoloration (bleaching of yellow color), which can be monitored by spectrophotometry. The method used was developed by Marco and modified by Öztürk et al. for use in 96-well microplates [69–70]. To prepare the emulsion, β-carotene (0.5 mg) was dissolved in 1 mL of chloroform and then added to linoleic acid (25 μL) and Tween 40 (200 mg). Chloroform was removed under vacuum and 50 mL of distilled water was added. The emulsion was vigorously shaken to incorporate the oxygen. The absorbance of the β-carotene emulsion was adjusted to between 0.8 and 0.9 at 470 nm by adding hydrogen peroxide. To 40 μL of FPE (2 mg/mL), 160 μL of the emulsion was added and the absorbance was immediately measured at 470 nm (t = 0 min) and at 30 min intervals for 2 h during incubation at 45 °C. Ascorbic acid was used as the standard. The bleaching rate of β-carotene was calculated using the following equation:

$$R = \frac{\ln \frac{A_o}{A_t}}{t}$$

Where R is the bleaching rate of β-carotene, A_0 is the absorbance at t = 0 min and A_t is the absorbance at time t = 120 min.

The antioxidant activity (%) of extracts was calculated using the following equation:

$$\text{Antioxidant activity}\ (\%) = \frac{R_c - R_e}{R_c} \times 100$$

Where R_c is the bleaching rate of control (distilled water) and R_e is the bleaching rate of the extract.

UV-photoprotective activity

Spectral characteristics. The spectrophotometric absorption of the extracts (10 mg/mL) and citric acid (4.75 mg/mL) was measured in a quartz cuvette at wavelengths ranging from 200–700 nm using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The maximum wavelength, which corresponded to the highest absorbance recorded for each sample, was determined from the absorbance versus wavelength plots.

Determination of the SPF. The FPE SPF was evaluated using the method described by Mansur et al. [71]. Briefly, 200 μL of extract at different concentrations (2, 5, 10, and 25 mg/mL) and the corresponding citric acid content (0.95, 2.38, 4.75 and 11.88 mg/mL) were placed into 96-well microplates. The absorbances were recorded between 290–320 nm at 5 nm increments. Five replicates were used for each test. SPF values were calculated using the following formula:

$$SPF_{value} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times A(\lambda)$$

Where CF is the correction factor equal to 10, λ is the wavelength, A is the absorbance at λ, EE is the erythemal effect spectrum and I is the solar-intensity spectrum. The values of EE(λ) × I(λ) were constant for each wavelength, as defined by Sayre et al. [72] (Table 1).

Table 1 Normalized product function used in the SPF calculation

Wavelength λ (nm)	EE × I (λ) constant
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

SPF, sun protection factor; EE, erythemal effect spectrum; I, solar-intensity spectrum.

Brine shrimp toxicity assay. The toxicity of FPE was evaluated using the brine shrimp lethality assay, as described by Meyer et al., with some modifications [59]. Extracts (concentrations between 0.39 and 25 mg/mL) and citric acid (between 0.18 and 11.88 mg/mL) were

prepared in distilled water. Two grams of brine shrimp eggs (San Francisco Bay Brand, Inc., Newark, CA, USA) were hatched in one liter of seawater in an incubator (Panasonic Cooled incubator, MIR-154-PE, Gunma, Japan) at 27 °C under constant light and oxygen supply. After approximately 36 h, the nauplii were collected in a Petri dish using a pipette to separate them from the shells and eggs that had not hatched. Ten moving larvae were placed in each well of the microplate containing 100 µL of seawater, and then 20 µL of FPE or citric acid was added. The volume of the well was set to 200 µL with seawater. Each assay was performed in triplicate. Potassium dichromate was used as the standard. The microplates were incubated in the incubator at 27 °C (light on). After 24 h, the surviving shrimp (those that showed movement) were counted in each well, and the death percentage was determined using the following formula:

$$Mortality\ (\%) = \frac{(SL_c - SL_e)}{SL_c} \times 100$$

Where SL_c is the number of surviving larvae in the control (without extract), SL_e is the number of surviving larvae in the presence of the extract.

The 50% lethal concentration of brine shrimp was determined from mortality data using probit analysis.

Statistical analysis

Results are expressed as mean ± standard deviation. Analysis of variance followed by Tukey's honestly significant difference multiple comparisons of means was used to detect differences between the extracts. Dunnett's bilateral statistical test was used to compare the results of extracts with those of the standard at a confidence interval of 95%. Probit analysis was performed using XLSTAT software (version 2014.5.03, Addinsoft, Damrémont, France). Statistical significance was set at $P < 0.05$.

Results

Phytochemical contents

Fig peels contained high amounts of phenolic compounds (between 3.85 and 8.63 g/100 g) and flavonoids (between 2.17 and 5.05 g/100 g), including the classes of flavonols (from 1–3.74 g/100 g) and anthocyanins (from 72–622 mg/100 g) (Figure 2). *Azenjer* had the highest phytochemical content, followed by *Tazegaght*. A significant variation in the phytochemical content ($P < 0.05$) was found among the four cultivars.

Antioxidant activity

Figure 3 presents the inhibitory activity of FPEs on β-carotene bleaching after 120 min of the reaction. The results showed similar graph evolution and slight differences between cultivars, indicating comparable inhibitory effects on β-carotene bleaching. At a concentration of 2 mg/mL, the extracts displayed good inhibition (86.25–88.3%). *Azenjer* extract showed the highest activity compared to the other cultivars at the same concentration (92.31%). Citric acid control also exhibited a noticeable inhibition (77.18%) at a concentration of 0.95 mg/mL (the concentration that corresponds to the citric acid content of FPE at 2 mg/mL), indicating that the presence of citric acid in extracts contributed to their overall activity. The ascorbic acid standard maintained the initial absorbance of the β-carotene solution for 120 min, indicating that it provided the highest protection against oxidation. At 0.8 mg/mL, ascorbic acid showed 80.53% inhibition. Interestingly, *Azenjer* extract at 2 mg/mL exhibited a similar profile to ascorbic acid at 0.8 mg/mL, proving the efficacy of this natural extract in protecting β-carotene against oxidation. The half-maximal inhibitory concentration (IC_{50}) values of extracts ranged from 88.84 µg/mL (*Azenjer* extract) to 107.83 µg/mL (*Bakor Noir* extract), significantly higher than that of the ascorbic acid standard (43.16 µg/mL).

Antimicrobial activity

The results of the antimicrobial tests are summarized in Table 2. All FPE tested showed antibacterial activity against both the Gram-positive and Gram-negative groups, with Gram-positive strains being more sensitive to the inhibitory action. Among the bacterial strains tested, *Bacillus subtilis* was the least resistant to the action of extracts (between 24.5 and 29.5 mm), while *Escherichia coli* was the most resistant (between 10.33 and 12.83 mm). The antibacterial action of *Azenjer* extract was significantly higher than that of the other extracts, except against *Escherichia coli* and *Pseudomonas aeruginosa*, where *Tazegaght* activity was significantly higher. Citric acid also exhibited moderate activity against all tested bacteria (Table 2), with the best action on *Bacillus subtilis*, indicating that citric acid contributed to the inhibitory activity of the extracts. Nevertheless, none of the FPEs showed higher antibacterial activity than antibiotics. Cefazolin was significantly more efficient against the two groups of bacteria tested in this study. In contrast, the FPEs did not show any noticeable antifungal activity.

Extracts that showed inhibition zones in the agar diffusion assay were further investigated to determine their MIC values. The results are presented in Table 3. The MIC values obtained consolidate the findings of the agar diffusion test, as smaller values were observed against *Bacillus subtilis* (156.25 µg/mL), followed by *Staphylococcus aureus* (312.5 µg/mL). Among the Gram-negative strains, *Pseudomonas aeruginosa* appeared to be more sensitive to *Azenjer* and *Bouankik* extracts (312.5 µg/mL). The results also showed that citric acid effectively inhibited the growth of all the tested bacteria, with MIC values of 74.22 µg/mL for *Bacillus subtilis* and 296.87 µg/mL for the other strains.

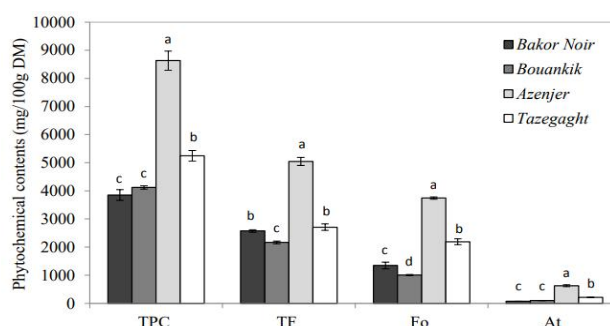

Figure 2 Phytochemical composition of FPE. FPE, fig peel extracts; TPC, total phenolic compounds content; TF, total flavonoids content; Fo, flavonols content; At, anthocyanins content; DM, dry matter. Vertical bars present standard deviations (n = 3). For each parameter, different letters (a > b > c > d) indicate significant differences ($P < 0.05$) by Tukey's honestly significant difference test.

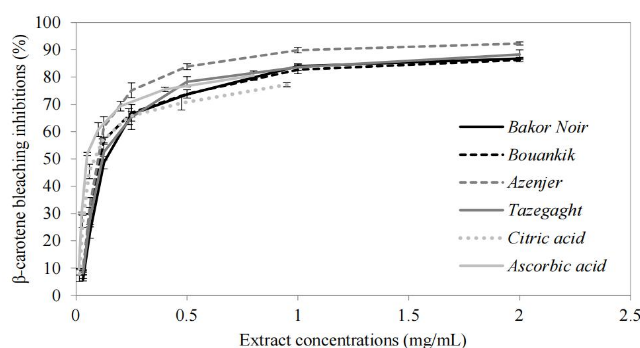

Figure 3 Antioxidant activity of FPE using the β-carotene/linoleic acid system. The X-axis represents extract concentrations expressed in mg/mL and the Y-axis is the β-carotene bleaching inhibitory activity of extracts expressed in percentage. The growing curve indicates that the higher the concentration of extracts, the higher the bleaching inhibitory activity, until reaching a given concentration where the maximum inhibitory effect is achieved. Vertical bars present standard deviations (n = 3). FPE, fig peel extracts.

Table 2 Antimicrobial activity of FPE, citric acid and antibiotic by agar well diffusion assay against pathogenic bacteria and fungi

	Inhibition zones (mm)							
	Gram-(+)			Gram-(−)			Fungi	
	Bacillus subtilis	*Listeria monocytogenes*	*Staphylococcus aureus*	*Salmonella enteritidis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus brasiliensis*
Bakor Noir	27.33 ± 0.58^{b}	15.83 ± 0.76^{b}	14.17 ± 1.04^{a}	13.67 ± 0.58^{b}	11.67 ± 0.58^{ab}	11.67 ± 0.58^{bc}	0.00	0.00
Bouankik	25.33 ± 0.76^{c}	14.17 ± 1.04^{b}	13.83 ± 0.76^{a}	16.33 ± 1.53^{ab}	10.33 ± 0.58^{b}	11.83 ± 0.76^{b}	0.00	0.00
Azenjer	29.50 ± 0.50^{a}	19.50 ± 1.50^{a}	14.33 ± 0.58^{a}	18.50 ± 2.18^{a}	11.83 ± 0.76^{ab}	11.33 ± 0.58^{bc}	0.00	0.00
Tazegaght	24.50 ± 0.50^{c}	15.67 ± 0.58^{b}	13.50 ± 0.50^{a}	15.83 ± 1.61^{ab}	12.83 ± 0.76^{a}	14.50 ± 0.50^{a}	0.00	0.00
Citric acid	16.17 ± 0.29^{d}	9.17 ± 1.04^{c}	9.67 ± 0.58^{b}	8.83 ± 1.04^{c}	9.83 ± 1.04^{b}	9.67 ± 1.15^{c}	0.00	0.00
Cefazolin	52.50 ± 0.50*	36.67 ± 1.53*	32.33 ± 1.26*	34.67 ± 0.58*	35.33 ± 1.15*	37.67 ± 1.53*	NT	NT

Results are presented as mean ± standard deviation (n = 3). Inhibition zones include the well (7 mm). Different letters (a > b > c > d) indicate significant differences (P < 0.05) between the extracts for each bacterial strain by Tukey's honestly significant difference test. *, Result of Cefazolin significantly different from all fig peel extracts (Dunnett's test, P < 0.05). FPE, fig peel extracts; NT, not tested.

Table 3 MIC of FPE, citric acid and standard antibiotic (Cefazolin) against Gram-positive and Gram-negative pathogenic bacteria

	MIC of extracts (μg/mL)					
	Gram-positive			Gram-negative		
	Bacillus subtilis	*Listeria monocytogenes*	*Staphylococcus aureus*	*Salmonella enteritidis*	*Escherichia coli*	*Pseudomonas aeruginosa*
Bakor Noir	156.25	625.00	312.50	625.00	625.00	625.00
Bouankik	312.50	625.00	625.00	625.00	625.00	312.50
Azenjer	156.25	625.00	312.50	312.50	625.00	312.50
Tazegaght	156.25	625.00	312.50	625.00	625.00	625.00
Citric acid	74.22	296.87	296.87	296.87	296.87	296.87
Cefazolin	39.06	78.12	156.25	312.50	78.12	39.06

MIC, minimal inhibitory concentration; FPE, fig peel extracts.

UV-Photoprotective activity

Figure 4 shows the absorption spectra of FPEs and citric acid at 10 and 4.75 mg/mL, respectively. The FPEs showed overall comparable profiles in three absorption ranges: bands of absorption in the UV-C region (between 200 and 290 nm), bands in the UV-A region (between 320 and 400 nm), and bands in the visible region (between 450 and 600 nm). The differences in absorbance intensity between the FPEs were recorded. *Azenjer* extract exhibited the highest intensity profile in the three absorption domains cited above, with maximum absorptions at wavelengths of 236, 331 and 513 nm. *Bakor Noir* extract presented high UV absorption spectra but low absorption in the visible domain, with maximums at 224, 327 and 513 nm. While *Bouankik* and *Tazegaght* extracts showed similar profiles with less intense absorption than the other extracts, the maximal absorbance values were found at 254, 330 and 508 nm. In contrast, citric acid exhibited a flat profile with no specific absorption characteristics, except for the band obtained at 222 nm. In general, the FPE demonstrated high absorption in the UV domain, indicating a potential protective effect against UV rays.

To evaluate the photoprotective activity of the extracts, the SPF was calculated for different concentrations of each FPE (2, 5, 10 and 25 mg/mL) in the UV-B domain (between 290 and 320 nm). Figure 5 summarizes the main results of the photoprotective activity. SPF values increased with increasing extract concentration and for a given concentration, significant differences were observed between the four extracts. The highest SPF value (28) was recorded for the *Azenjer* extract at 25 mg/mL; the other extracts exhibited SPF values ranging from 12–16 at the same concentration. The SPF values calculated for citric acid (all concentrations tested) and the extracts at 2 and 5 mg/mL were lower than 7.

Brine shrimp toxicity

The results of brine shrimp toxicity tests are presented in Table 4. All FPEs exhibited toxicity towards brine shrimp larvae at concentrations higher than 781.25 μg/mL, and mortality rates increased from 0–93.33% with the increase in concentration. Lethal concentration of

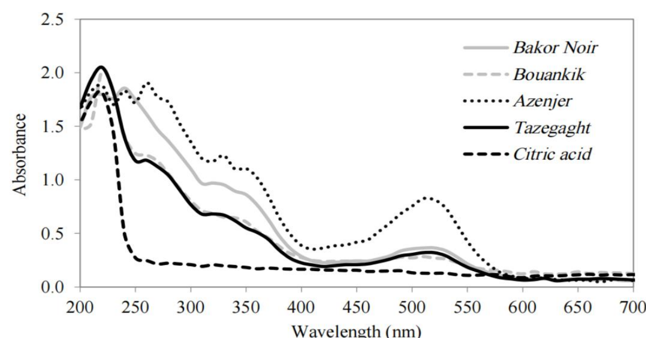

Figure 4 Absorption spectra of FPE and citric acid between 200 and 700 nm. The X-axis is the wavelengths used in the spectrophotometric scanning expressed in nanometers, and the Y-axis represents the absorbance values of each extract at the considered wavelengths. FPE, fig peel extracts.

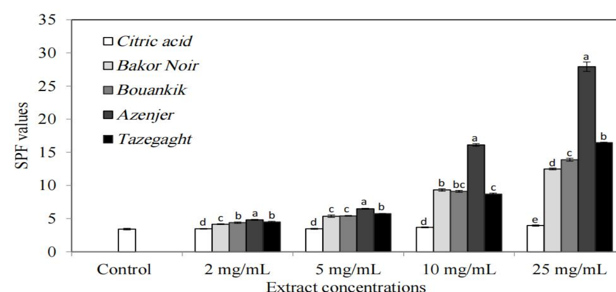

Figure 5 SPF values of FPE at different concentrations (2, 5, 10 and 25 mg/mL). Vertical bars present standard deviations (n = 3). The control consists of distilled water. Different letters (a > b > c > d > e) indicate significant differences between extracts at the same concentration (P < 0.05) by Tukey's honestly significant difference test. SPF, sun protection factor; FPE, fig peel extracts.

50% (LC_{50}) values differed slightly among the extracts; the lowest value was recorded for *Azenjer* extract (1,976.96 μg/mL). Potassium dichromate, used as a positive standard, was highly toxic to brine shrimp larvae (LC_{50} of 21.69 μg/mL) compared to the FPEs. Citric acid also exhibited a toxic effect on brine shrimp larvae, with an LC_{50} value of 922.02 μg/mL.

Discussion

Cosmeceuticals combine the properties of cosmetics, which are applied on the surface of the skin for a purely aesthetic effect and pharmaceuticals, which provide healing of skin problems. At this frontier, cosmeceuticals seem to treat skin problems gently and durably, without causing side effects. Scientific research to identify new active ingredients for innovative cosmeceutical formulations is in full swing. Research has mainly relied on plants as sources of sustainable and environmentally bioactive compounds. Among the promising sources, *F. carica* is a good candidate as it has been used as an efficient medicinal and cosmetic plant for a long time. In ancient Rome, figs were mixed with bananas, oat flour, and rose water to create a facial cream [73]. Scientific research has shown that a topical cream containing *F. carica* fruit extract at 4% applied to human volunteers for 8 weeks decreased skin melanin and sebum and increased skin hydration significantly without causing any irritation [74]. In another study, a formulation containing *F. carica*, among other plant extracts, at 2% showed antioxidant and anti-collagenase activities and significantly reduced wrinkle depth, length and area by 11.5, 10.07 and 29.55%, respectively, after 56 days of treatment in human volunteers [75]. The phytochemicals in *F. carica* extract, including phenolic acids and flavonoids, enhance skin repair by diminishing oxidation and inflammation; thus, treatment with *F. carica* extract restores the epidermis and improves skin lightness [76]. In our previous investigation, flavonoid-rich *F. carica* peel extracts showed astonishing anti-tyrosinase, free radical scavenging and metal ion-chelating activities, indicating high anti-hyperpigmentation and antioxidant properties [47]. The flavonoid group contains thousands of active compounds, such as quercetin and catechin, which are widely used as antioxidants, anti-aging agents, and anti-inflammatory molecules, in addition to protecting against UV-induced skin cancer and DNA damage [77].

In the present study, peels from dark fig fruits were utilized to extract flavonoids, as these compounds are mostly concentrated in the external parts (peels). The procedure aimed to maximize the recovery of anthocyanins, an important class of flavonoids that are highly susceptible to degradation from environmental conditions such as temperature, light, and oxidation, and to extract flavonoids that present structural and chemical similarities to anthocyanins. All analyzed cultivars contained high phenolic and flavonoid contents. However, the two cultivars belonging to the Smyrna type (*Azenjer* and *Tazegaght*), characterized by thin peels with black or reddish-violet colors, presented the highest amounts. The phenolic contents found in the present study are in accordance with the results reported by Maghsoudlou et al. and Harzallah et al. for peels of the *Siyah* cultivar (8.08 g/100 g) and peels of the *Kohli* cultivar (7.42 g/100 g) [78–79]. However, lower levels have been reported in other studies [40, 41, 49]. The flavonoid content of fig peels reported in the literature is at least two times lower than our findings [41, 49, 78, 79]. The higher flavonoid content could be explained by the optimization step that improved extraction conditions, leading to the highest recovery of sensitive flavonoids [63]. In addition, fig genotypes, geographical and edapho-climatic conditions, and even orchard agricultural practices could modify flavonoid biosynthesis pathways, leading to significant differences in flavonoid contents [50, 80, 81].

The antioxidant activity of an extract is an important parameter when formulating a cosmeceutical product. Antioxidant molecules can reverse or suppress the harmful action of free radicals on cellular constituents via one or multiple mechanisms of action, including free radical scavenging, reducing oxidized molecules, binding metal ions and breaking chain reactions that generate lipid peroxyl radicals. The latter mechanism of action is particularly important for skin protection, given that the skin is often exposed to external inducers of oxidative chain reactions initiated by UV radiation that occur mainly in lipids, proteins and DNA [82]. To screen for extracts that break chain reactions, the -carotene-linoleic acid model simulated the natural lipid peroxyl radical generation system. The principle of the assay is to determine the capacity of extracts to neutralize free radicals resulting from the oxidation of fatty acids. In the presence of oxygen, linoleic acid in the emulsion is oxidized to form hydroperoxides, which are free radicals formed upon the removal of a hydrogen atom from one of the diallylic methylene groups of linoleic acid [83]. In the presence of hydroperoxides, β-carotene undergoes a bleaching process induced by the oxidation of its unsaturated bonds, resulting in the loss

Table 4 Results of the brine shrimp cytotoxicity of FPE and citric acid

	Concentration (μg/mL)	Initial number of larvae	Surviving larvae	Mortality rate (%)	LC_{50} (μg/mL)	Confidence interval at 95%	
						Inferior limit	Superior limit
Bakor Noir	3,125.00	30	5	83.33	2,459.59	2,160.76	2,758.19
	1,562.50	30	29	3.33			
	781.25	30	30	0.00			
Bouankik	3,125.00	30	7	76.67	2,281.55	1,957.24	2,670.82
	1,562.50	30	26	13.33			
	781.25	30	29	3.33			
Azenjer	3,125.00	30	2	93.33	1,976.96	1,718.38	2,288.41
	1,562.50	30	25	16.67			
	781.25	30	29	3.33			
Tazegaght	3,125.00	30	7	76.67	2,487.81	2,179.97	2,832.68
	1,562.50	30	28	6.67			
	781.25	30	30	0.00			
Citric acid	1,485.00	30	0	100.00	922.02	812.61	1,062.77
	742.50	30	27	10.00			
	371.25	30	29	3.33			
Potassium dichromate	40.00	30	5	83.33	21.69	18.38	25.51
	20.00	30	14	53.33			
	10.00	30	29	3.33			

FPE, fig peel extracts; LC_{50}, lethal concentration of 50%.

of its characteristic color. However, β-carotene bleaching can be avoided or, at least, reduced by the presence of active antioxidants that neutralize the free radicals and stop the chain reactions.

The capacity of FPEs as chain-breaking antioxidants was confirmed in the present study. The antioxidant activity of *F. carica* extracts using the β-carotene/linoleic acid system has been reported in several studies. The fig syrup's ability to inhibit lipid peroxidation evaluated by Puoci et al. using the β-carotene/linoleic acid system was [46]. They found the IC_{50} value to be 2.04 mg/mL, while whole fig fruit extract exhibited an IC_{50} value equivalent to 0.24 mg/mL [36]. Besides, in a study on peels of green Portuguese fig (*Pingo de Mel*), Palmeira et al., found that 50% β-carotene bleaching inhibition was reached at the concentration of 135 μg/mL [50]. This value was higher than our results, confirming that dark fig extracts are more effective antioxidants and contain strong bioactive compounds.

According to Marrelli et al., the β-carotene bleaching assay is more suitable for detecting higher lipophilicity antioxidants that probably have more biological relevance because these scavengers are more likely to act in biological membranes, which are the major targets of oxidative damage [15]. The polarity of phenolic compounds varies between different classes according to their chemical structure and the degree of substitution of their hydroxyl groups. Rice-Evans reported that for optimal inhibition of lipid peroxidation, a catechol group in the B-ring, a "2-3" double bond conjugated with the 4-oxo function, and 3- and 5-hydroxyl groups are agreed on structural criteria [84]. According to Hassimotto et al., when the antioxidant activity of anthocyanidins in the liposome system was compared, the higher activity was related to a lower polarity, which suggests that among anthocyanidins, malvidin showed the best inhibition due to its low polarity conferred by the substitution of the 3',5'-dihydroxyl groups of the B-ring with methoxyl groups [85]. The rules that determine the behavior of one class of flavonoids in a system do not apply to another class. In addition to appropriate structural arrangements for effective hydrogen donation to lipid peroxyl radicals, the antioxidant activity of phenolic compounds depends on the lipophilicity and accessibility of the antioxidant to the lipid radicals to be scavenged and the oxidation system, degree of glycosylation, partition coefficient and concentration of the antioxidant [84, 85]. When non-polar compounds show strong antioxidant activity in emulsions, scientists call this phenomenon the "polar paradox", explained by the concentration of antioxidants at the lipid/air interface, ensuring the protection of the emulsion against oxidation [86].

In addition to the capacity of FPEs to inhibit the generation of lipid radicals, extracts have also been tested previously for their antioxidant activity by exploring other mechanisms of action, including free radical scavenging, reducing power and metal chelating capacity. The FPEs were highly efficient as free radical scavenging antioxidants and also showed good copper binding capacity, strongly correlated with the inhibitory activity of tyrosinase, which is a target enzyme in depigmentation treatments and melanogenesis regulation in human skin [47].

The skin constitutes an effective barrier against UV radiation; however, excessive exposure causes dermal alterations and photoaging. UV rays considerably affect the skin in the short and long term. UV-B (290–320 nm) causes DNA damage and induces pigmentation changes and sunburn cells, which enhances carcinogenesis, whereas UV-A (320–400 nm) penetrates deeper into the dermis and generates reactive oxygen species that accelerate skin deterioration and aging [82]. Applying sunscreens to the skin could prevent damage related to UV exposure. Sunscreens contain two categories of UV filters: UV radiation absorbers and blockers that reflect UV radiation [56]. The trend in sunscreen formulations is the addition of plant antioxidants such as retinoids (vitamin A), ascorbic acid (vitamin C), resveratrol, caffeine, tocopherols (vitamin E), cinnamates, polyphenols and specific extracts (algae, licorice, *Matricaria chamomilla Echinacea pallida Polypodium leucotomos* and *Senna alata*) that show broad-spectrum photoprotective capabilities [56]. Such extracts may contain flavonoids that exhibit antioxidative properties because of their structural features.

All phenolic compounds absorb in the UV region, and two absorption bands are specifically characteristic of flavonoids: Band I (maximum absorption between 300 and 550 nm) originates from aromatic ring A, and Band II (maximum absorption between 240 and 285 nm) originates from ring B. Among the flavonoid subgroups, anthocyanins and anthocyanidins are characterized by typical absorption spectra in the visible range (maximum absorption between 500 and 530 nm), while phenolic acids and hydroxycinnamic acids show maximum absorption in the range of 230–330 nm [87]. The absorption profiles obtained in the present study indicate that FPEs are composed of the same groups of compounds, with individual differences in their structures and concentrations. Considering the presented data, the absorption characteristics of our extracts matched the absorption profile of flavonoids, confirming the results of the phytochemical analysis. In addition to flavonoids, FPEs can contain considerable amounts of phenolic acids, as concluded from the absorption spectra in the UV domain. Phenolic acids could be free compounds or acylating moieties of anthocyanins.

The photoprotective activity of the extracts was evaluated by SPF evaluation at four different FPE concentrations in the UV-B range (between 290 and 320 nm). The in vitro method used in the present investigation correlates well with in vivo tests. It relates the sample absorbance to the erythematogenic effect of radiation and the UV-B light intensity at specific wavelengths [88]. SPF is a simple way to quantify the efficacy of a sunscreen formulation in protecting the skin from damage caused by UV radiation. SPF is defined as the UV energy required to produce a minimal erythemal dose (MED) on protected skin divided by the UV energy required to produce an MED on unprotected skin, where MED is defined as the lowest time interval or dosage of UV irradiation sufficient to produce perceptible erythema on unprotected skin [89]. High SPF values indicate increased protection against skin damage. Generally, SPF values are categorized in the 6–10, 15–25, 30–50, or 50+ range, corresponding to low, medium, high and very high protection levels [90]. Considering the obtained data, FPEs at 25 mg/mL were able to confer medium protection against UV-B radiation. However, the extracts showed a low effect at 10 mg/mL. According to the Food and Drug Administration (USA), SPF values equal to or greater than 15 are appropriate for pharmaceutical preparations with photoprotective activity [91]. Among the FPEs studied, *Azenjer* extract at 10 and 25 mg/mL and *Tazegaght* extract at 25 mg/mL could be potential candidates for use in sunscreen preparations.

Citric acid results showed that the photoprotective effect exhibited by FPEs at high concentrations was essentially attributable to the phenolic fractions and specifically to the flavonoids that showed absorption capacity in the UV region. Many authors have associated the photoprotective activity of plant extracts with flavonoid content, supporting this result [91–93]. Flavonoid compounds, such as apigenin, luteolin, artocarpin, rutin and cyanidin-3-glucoside, found in fruit extracts, confer skin photoprotection through different mechanisms, including absorption of UV rays, inhibition of UV-induced oxidative stress (antioxidant activity), reduction of erythema and inflammation (anti-inflammatory activity), enhancement of epidermal thickening, reduction of DNA damage and modulation of several signaling pathways [94]. To the best of our knowledge, this is the first time *F. carica* extracts was investigated for their photoprotective activity against UV radiation. Given the promising results obtained, extracts with high flavonoid and At (*Azenjer* and *Tazegaght* extracts) are worth incorporating into sunscreen cream formulations that protect against both UV-A and UV-B radiation.

The FPEs were assayed to determine their toxicity levels using brine shrimp larvae. The brine shrimp (*Artemia salina*) lethality assay is a useful method to assess the toxic potential of plant extracts because of several advantages, including its simplicity, rapidness, inexpensiveness, high sensitivity and repeatability [61]. The test consisted of in vitro culturing of a precise number of larvae in a medium containing a known concentration of extracts and counting the number of surviving larvae after a period of incubation not

exceeding 48 h. A LC_{50} was estimated by probit analysis that fits the dose-response experiments. The analysis goes through the conversion of mortality rates (%) into probability units (probits) with the aid of Finney's table [95], graphing the probits versus the log of extract concentrations and then fitting a linear regression. The LC_{50} is equal to the inverse log of the concentration corresponding to a probit of 5.00. According to Meyer et al., extracts with LC_{50} above 1,000 μg/mL are considered non-toxic and those with LC_{50} less than 1,000 μg/mL are considered toxic [59]. Potassium dichromate, used as a standard, was highly toxic, citric acid was slightly toxic, and FPEs were non-toxic. The toxic effect observed with citric acid could be explained by the decrease in pH of the growth medium, which could also explain the brine shrimp mortality that occurred in contact with extracts containing citric acid. In fact, brine shrimps grow naturally in seawater where the pH is equal to 8.0 ± 0.5 and when pH decreases to values below 8, the growth of larvae is significantly affected. Generally, the pH of the medium is adjusted using sodium hydroxide or sodium carbonate to ensure egg hatching and avoid lethality caused by pH decrease during incubation [61]. However, in our case, citric acid was added intentionally to extracts to stabilize the bioactive anthocyanins and prevent their degradation. Therefore, citric acid became an important part of the chemical composition of extracts and could not be neutralized. This is why a citric acid control was added alongside extracts and standards in all our experiments.

The long history of ethnomedicinal applications of *F. carica*, with no reports of any side effects, suggests that it could be considered safe [52]. Furthermore, acute toxicity studies of *F. carica* extracts using animal models (albino mice and rats) by Patil and Patil Sruthi et al. reported no signs of toxicity even at doses of 5,000 and 2,000 mg/kg of body weight, respectively [28, 96]. This is the first time that *F. carica* extracts have been assayed for brine shrimp toxicity, and the results support previous findings on extract safety. Comparing the LC_{50} obtained for figs to other fruit extracts using the brine shrimp assay, it is clear that fig extracts are by far less toxic. Pomegranate fruit rind (*Punica granatum*) and lemon peel (*Citrus medica var. limon*) exhibited LC_{50} values equal to 45 and 92.5 μg/mL, respectively and methanolic extracts of red raspberry fruits (*Rubus rosifolius*) showed LC_{50} values superior to 500 μg/mL [97, 98]. This could be attributed to the phytochemical composition of the extracts being dominated by flavonoids of different classes. Flavonoids are considered safe and non-toxic because they are widely distributed in edible plants and beverages and have been used in traditional medicine, pharmaceutical products and dietary supplements without any side effects [53]. In addition, a toxicological study on a flavonoid-rich extract from buckwheat containing high levels of rutin and quercetin showed that continuous feeding of mice for 30-days had no toxic effect on development, hematology indexes, biochemistry and pathology [99]. Therefore, FPEs can be considered non-toxic and safe for use in food, pharmaceutical and cosmeceutical products.

Regarding antimicrobial activity, an agar well diffusion assay was applied to screen the capacity of FPEs to inhibit the growth of six pathogenic bacterial strains belonging to Gram-positive and Gram-negative groups and to test their antifungal activity. Inhibition of microbial growth occurred when a clear zone appeared around the well containing the extract. The sensitivity of a microbial strain to the extract was evaluated by the diameter of the inhibition zone. A larger inhibition zone indicates a higher antimicrobial action. The present work showed that FPEs possess antibacterial activity against Gram-positive and Gram-negative bacteria, but no antifungal activity was observed. Aligiannis et al. proposed a classification of the antibacterial action of plant extracts into three categories based on MIC values: (1) strong inhibition when MIC is less than 500 μg/mL; (2) moderate inhibition when MIC is between 600 and 1,500 μg/mL; and (3) weak inhibition when MIC exceeds 1,600 μg/mL [100]. According to this classification, all the FPEs tested here are strong *Bacillus subtilis* and *Staphylococcus aureus* inhibitors. FPEs can be classified as strong to moderate antibacterial agents for the other strains.

The present results concur with the findings of Palmeira et al. on the peel extracts of green fig cultivars [50]. The authors tested five Gram-positive and four Gram-negative bacteria. They reported that peel extracts showed antibacterial activity against the two groups, with more effectiveness against Gram-positive bacteria, but found higher MIC values (the most sensitive strain was *Staphylococcus aureus* with MIC of 2.5 mg/mL), indicating that dark FPE are more effective antibacterial agents than green peel extracts. The antimicrobial activity of aqueous FPE was also studied by Oliveira et al.; however, they found no inhibitory effect against *Bacillus cereus Staphylococcus aureus Staphylococcus epidermidis*, *Escherichia coli*, or *Pseudomonas fluorescens* [101]. The antimicrobial effect of fig fruit extracts has been previously reported. Dried *Azenjer* fruit extracts were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and other strains by Debib et al. [37]. The methanolic extract showed inhibitory effects against *Bacillus subtilis* (MIC of 128 μg/mL) and *Pseudomonas aeruginosa* (MIC of 64 μg/mL), but no inhibition was recorded against the other strains. Conversely, the authors recorded the best inhibitory activity against the *Candida albicans* yeast (MIC of 32 μg/mL). The high sensitivity of *Bacillus subtilis* towards *F. carica* fruit extracts was also pointed out by Soni et al. and Shahbazi [36, 43]. In addition, *F. carica* fruit extracts were verified to be highly efficient against *Pseudomonas aeruginosa* (MIC of 0.94 μg/mL) and *Salmonella* species (MIC of 1.87 μg/mL) and are potential candidates for designing drugs with broad-spectrum antibiotic activity [102]. Our results are in agreement with the literature regarding the antibacterial activity of *F. carica* extracts but diverge in the antifungal effect.

The observed activity could be attributed to the synergistic action of citric acid and the phytochemical compounds present in the extracts. Organic acids such as citric acid are commonly used as preservatives owing to their antibacterial effect. Ashour et al. found that citric acid completely inhibited *Staphylococcus aureus* and *Escherichia coli* growth with MICs equal to 25 and 50 μg/mL, respectively [103]. Organic acids affect microorganisms by lowering the cytoplasmic pH below the optimal value for their viability and interfering with many enzymatic processes, causing an increase in the osmotic stress that results in the collapsing of the cells [104]. Organic acids such as citric and lactic acids also function as permeabilizers of the Gram-negative bacterial outer membrane and consequently increase the effects of other antimicrobial substances [105]. Given that the citric acid fraction in the extracts is approximately eight times higher than the phenolic fraction (extracts at 20 mg/mL contain between 0.76 and 1.72 mg of phenolic compounds against 9.5 mg of citric acid), the antibacterial action of citric acid remains weak compared to the phenolic activity.

The main bioactive compounds recognized as antimicrobial agents are polyphenols, polypeptides, terpenoids, flavonoids, isothiocyanates, tannins, lectins and alkaloids [106]. As peel extracts are a mixture of compounds, it is difficult to identify the molecules responsible for the antimicrobial effects; however, in the FPE tested, flavonoids represent the major bioactive compounds likely to confer antimicrobial action. Flavonoids are synthesized by plants in response to microbial infection; therefore, it is not surprising that they show antimicrobial activity against a wide group of microorganisms [107]. The antimicrobial activity of flavonoids is based on different mechanisms of action: (1) inhibition of nucleic acid (DNA and RNA) synthesis, (2) alteration of cytoplasmic membrane synthesis and function, (3) inhibition of energy metabolism, (4) reduction in cell attachment and biofilm formation, (5) reduction of pathogenicity, (6) inhibition of porins on the cell membrane and (6) alteration of membrane permeability [108, 109]. The metal-chelating capacity of flavonoids could also be considered an important mechanism of action against pathogenic bacteria [104]. The extent of the antimicrobial activity of flavonoids depends on their structural characteristics that confer hydrophobicity to the flavonoid molecule, namely the substitution of aromatic rings [108]. For example, flavones that possess at least one hydroxyl group in ring A and another position (such as C-5 and C-6) were found to exhibit strong antibacterial activity. However, methoxylation at C-7 decreased the activity and hydroxylation at positions 5 and 7 in ring A is important for the antibacterial activity of flavonols, as well as hydroxylation of B and C

rings [109]. Anthocyanins, which are more hydrophilic molecules, are reported to inhibit Gram-negative bacteria but not Gram-positive bacteria [110]. This selective action was explained by the differences in cell wall structure between the two groups of bacteria, in which the outer membrane of Gram-negative bacteria acts as a preventive barrier against hydrophobic compounds but not against hydrophilic compounds [111]. It is also explained also by the ability of anthocyanins to induce the release of lipopolysaccharide molecules from the outer membrane of Gram-negative bacteria. Therefore, the high At of *Azenjer* extract could explain the results obtained for Gram-negative strains.

Conclusion

The present study tested peel extracts from dark figs were tested for their cosmeceutical aptitudes (antioxidant, UV absorption, and SPF) and pharmacological activities against pathogenic bacteria. The results showed that extracts with high flavonoid contents could effectively inhibit lipid peroxidation. Among the studied cultivars, *Azenjer* and *Tazegaght* extracts exhibited the best results. All FPE presented high absorption in the UV domain and acceptable SPF values; thus, these extracts could be incorporated into sunscreen preparations. However, additional research is needed to test the stability of extracts in emulsion systems and their synergistic effect with other molecules to increase the SPF values before in vivo testing. Considering the strong antibacterial activity and toxicity results, *F. carica* peel extracts could be considered as original safe ingredients in the formulation of new antibacterial drugs. Further research is required to identify active compounds and their mechanisms of action against pathogens.

References

1. Chawla A, Kaur R, Sharma AK. *Ficus carica* Linn.: a review on its pharmacognostic, phytochemical and pharmacological aspects. *Int J Pharm Phytopharm Res*. 2012;1(4):215–232. https://www.researchgate.net/publication/236154374_
2. Azwanida N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants*. 2015;4(3):1000196. https://doi.org/10.4172/2167-0412.1000196
3. Lansky EP, Paavilainen HM, Pawlus AD, Newman RA. *Ficus* spp. (fig): ethnobotany and potential as anticancer and anti-inflammatory agents. *J Ethnopharmacol*. 2008;119(2): 195–213. https://doi.org/10.1016/j.jep.2008.06.025
4. Kahrizi D, Molsaghi M, Faramarzi A, et al. Medicinal plants in holy Quran. *Am J Sci Res*. 2012;42:62–71. https://www.researchgate.net/publication/234659258_
5. Lansky EP, Paavilainen HM. *Figs: The Genus Ficus*. Boca Raton, Florida, USA: CRC Press;2010. https://doi.org/10.1201/9781420089677
6. Flaishman MA, Rodov V, Stover E. The fig: botany, horticulture, and breeding. *Hortic Rev*. 2008;34:113–197. https://doi.org/10.1002/9780470380147.ch2
7. Martínez GJ. Natural pharmacopoeia and treatment of skin conditions in the traditional medicine of the peasants of the mountains of Córdoba. *Dominguezia*. 2008;24(1):27–46. (Argentina) http://www.dominguezia.org/volumen/articulos/2413.pdf
8. Arafa EA, Hassan W, Murtaza G, Buabeid MA. *Ficus carica* and *Sizigium cumini* regulate glucose and lipid parameters in high-fat diet and streptozocin-induced rats. *J Diabetes Res*. 2020; 6745873:1–9. https://doi.org/10.1155/2020/6745873
9. Gillani M, Iftikhar H, Lodhi A, Pasha I. Development and characterisation of fig (*Ficus carica*) leaves tea with special reference to hypoglycemic effect. *Pak J Sci Ind Res*. 2018; 61(2):78–83. https://doi.org/10.52763/PJSIR.BIOL.SCI.61.2.2018.78.83
10. Irudayaraj SS, Christudas S, Antony S, Duraipandiyan V, Al-Dhabi NA, Ignacimuthu S. Protective effects of *Ficus carica* leaves on glucose and lipids levels, carbohydrate metabolism enzymes and β-cells in type 2 diabetic rats. *Pharm Biol*. 2017;55(1):1074–1081. https://doi.org/10.1080/13880209.2017.1279671
11. Purnamasari R, Winarni D, Permanasari AA, Agustina E, Hayaza S, Darmanto W. Anticancer activity of methanol extract of *Ficus carica* leaves and fruits against proliferation, apoptosis, and necrosis in Huh7it cells. *Cancer Inform*. 2019;18: 1176935119842576. https://doi.org/10.1177/1176935119842576
12. Boyacioglu O, Kara B, Can H, Yerci TN, Yilmaz S, Boyacioglu SO. Leaf hexane extracts of two Turkish fig (*Ficus carica* L.) cultivars show cytotoxic effects on a human prostate cancer cell line. *Agric Food Sci Res*. 2019;6(1):66–70. https://doi.org/10.20448/journal.512.2019.61.66.70
13. Lightbourn AV, Thomas RD. Crude edible fig (*Ficus carica*) leaf extract prevents diethylstilbestrol (DES)-induced DNA strand breaks in single-cell gel electrophoresis (SCGE)/comet assay: literature review and pilot study. *J Bioequivalence Bioavailability*. 2019;11(2):19–28. https://doi.org/10.35248/0975-0851.19.11.389
14. Eteraf-Oskouei T, Allahyari S, Akbarzadeh-Atashkhosrow A, et al. Methanolic extract of *Ficus carica* Linn. leaves exerts antiangiogenesis effects based on the rat air pouch model of inflammation. *Evid Based Complement Alternat Med*. 2015;2015: 760405. https://doi.org/10.1155/2015/760405
15. Marrelli M, Menichini F, Statti GA, et al. Changes in the phenolic and lipophilic composition, in the enzyme inhibition and antiproliferative activity of *Ficus carica* L. cultivar Dottato fruits during maturation. *Food Chem Toxicol*. 2012;50(3–4): 726–733. https://doi.org/10.1016/j.fct.2011.12.025
16. Hashemi SA, Abediankenari S. Suppressive effect of fig (*Ficus carica*) latex on esophageal cancer cell proliferation. *Acta Fac Medicae Naissensis*. 2013;30(2):93–96. https://doi.org/10.2478/v10283-012-0042-2
17. Alsahli MA, Almatroudi A, Khan AA, Alhumaydhi FA, Alrumaihi F, Rahmani AH. *Ficus carica* (fig) fruit extract attenuates CCl_4-induced hepatic injury in mice: a histological and immunohistochemical study. *Int J Pharmacol*. 2019;15(3): 370–376. https://doi.org/10.3923/ijp.2019.370.376
18. Oyefuga OH, Alabi MA, Idowu G, et al. Influence of ethanolic and aqueous extract of the bark of *Ficus carica* on liver function enzymes of Wister white albino rat. *Scholarly J Biol Sci*. 2013;2(4):49–54. https://www.researchgate.net/publication/261097876_
19. Allahyari S, Delazar A, Najafi M. Evaluation of general toxicity, anti-oxidant activity and effects of *Ficus carica* leaves extract on ischemia/reperfusion injuries in isolated heart of rat. *Adv Pharm Bull*. 2014;4 (Suppl 2):577–582. https://doi.org/10.5681/apb.2014.085
20. Koka S, Barik R, Joshi J, Jain SK. Effect of *Ficus carica* fruit extract on experimentally induced inflammation and nociception. *J Pharm Phytother*. 2013;1(5):8–11. https://www.semanticscholar.org/paper/Effect-of-Ficus-carica-fruit-extract-on-induced-and-Koka-Barik/16be7344022708ed478f080b1f2a93f7e41a2880
21. Park S, Han J, Im K, Whang WK, Min H. Antioxidative and anti-inflammatory activities of an ethanol extract from fig (*Ficus carica*) branches. *Food Sci Biotechnol*. 2013;22(4):1071–1075. https://doi.org/10.1007/s10068-013-0185-7
22. Turkoglu M, Pekmezci E, Kilic S, Dundar C, Sevinc H. Effect of *Ficus carica* leaf extract on the gene expression of selected factors in HaCaT cells. *J Cosmet Dermatol*. 2017;16(4):e54–e58.

https://doi.org/10.1111/jocd.12344
23. Amol PP, Vikas VP, Vijay RP, Rajesh YC. Anthelmintic and preliminary phytochemical screening of leaves of *Ficus carica* Linn against intestinal helminthiasis. *Int J Res Ayurveda Pharm*. 2010;1(2):601–605. https://www.cabdirect.org/cabdirect/abstract/20113195429
24. Yang XM, Yu W, Ou ZP, Ma HL, Liu WM, Ji XL. Antioxidant and immunity activity of water extract and crude polysaccharide from *Ficus carica* L. fruit. *Plant Foods Hum Nutr*. 2009;64(2): 167–173. https://doi.org/10.1007/s11130-009-0120-5
25. Belguith-Hadriche O, Ammar S, Mar Contreras Md, et al. Antihyperlipidemic and antioxidant activities of edible Tunisian *Ficus carica* L. fruits in high fat diet-induced hyperlipidemic rats. *Plant Foods Hum Nutr*. 2016;71(2): 183–189. https://doi.org/10.1007/s11130-016-0541-x
26. Dayanand K, Kishanchandra K. Phytochemical investigation and evaluation of antihyperlipidemic activity of *Ficus carica* Linn. fruit extracts. *Global J Res Analysis*. 2012;3(1):63–64. https://doi.org/10.15373/22778160/June2014/63
27. Kore KJ, Shete RV, Kale BN, Borade AS. Protective role of hydroalcoholic extract of *Ficus carica* in gentamicin induced nephrotoxicity in rats. *Int J Pharm Life Sci Res*. 2011;2(8): 978–982. https://www.semanticscholar.org/paper/Protective-role-of-hydroalcoholic-extract-of-Ficus-Kore-Shete/ce4b731b607f4c626b73deccbdca21192f3026de
28. Sruthi B, Sunny G, Naz H, Sakthivel S. Diuretic activity of ethanolic extracts of *Ficus carica* L. fruits. *Int J Res Pharmacol Pharmacother*. 2012;1(1):23–26. https://journals.indexcopernicus.com/api/file/viewByFileId/410665.pdf
29. Munteanu M, Gligor R, Pogurschi E, Trifunschi S. Diuretic activity of *Ficus carica* L. extracts. Analele Universității din Oradea, Fascicula: Ecotoxicologie, Zootehnie și Tehnologii de Industrie Alimentară. 2016;15(A):75–82. http://protmed.uoradea.ro/facultate/publicatii/ecotox_zooteh_ind_alim/2016A/imapa/11Munteanau%20Melania.pdf
30. Lohar PS, Lohar MS, Roychoudhury S. Erythropoietic effects of some medicinal plants of India on experimental rat model. *Slovak J Anim Sci*. 2009;42(2):95–98. http://www.cvzv.sk/slju/09_2/Lohar.pdf
31. Raafat K, Wurglics M. Phytochemical analysis of *Ficus carica* L. active compounds possessing anticonvulsant activity. *J Tradit Complement Med*. 2019;9(4):263–270. https://doi.org/10.1016/j.jtcme.2018.01.007
32. Oh HG, Lee HY, Seo MY, et al. Effects of *Ficus carica* paste on constipation induced by a high-protein feed and movement restriction in beagles. *Lab Anim Res*. 2011;27(4):275–281. https://doi.org/10.5625/lar.2011.27.4.275
33. Chandra D, Kashyap P. Comparative in-vitro anthelmintic activity of different parts of *Ficus carica*. *Int J Herb Med*. 2011; 1(1):8–10. https://www.researchgate.net/publication/265672476_
34. Begum F, Shivakrishna P, Savya B, et al. Wound healing activity of methanolic leaf extract of *Ficus carica* in albino rats research article. *Int J Curr Res*. 2013;5(9):2631–2635. https://www.journalcra.com/sites/default/files/issue-pdf/Download%204007.pdf
35. Camero M, Marinaro M, Lovero A, et al. In vitro antiviral activity of *Ficus carica* latex against caprine herpesvirus-1. *Nat Prod Res*. 2014;28(22):2031–2035. https://doi.org/10.1080/14786419.2014.918120
36. Shahbazi Y. Antibacterial and antioxidant properties of methanolic extracts of apple (*Malus pumila*), grape (*Vitis vinifera*), pomegranate (*Punica granatum* L.) and common fig (*Ficus carica* L.) fruits. *Pharm Sci*. 2017;23(4):308–315. https://doi.org/10.15171/PS.2017.45
37. Debib A, Tir-Touil A, Mothana RA, Meddah B, Sonnet P. Phenolic content, antioxidant and antimicrobial activities of two fruit varieties of Algerian *Ficus carica* L. *J Food Biochem*. 2014; 38(2):207–215. https://doi.org/10.1111/jfbc.12039
38. Shafique F, Naureen U, Zikrea A, et al. Antibacterial and antifungal activity of *Ficus carica* plant extract. *J Pharm Res Int*. 2021;33(18):1–9. https://doi.org/10.9734/jpri/2021/v33i1831311
39. Mahmoudi S, Khali M, Benkhaled A, Benamirouche K, Baiti I. Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian *Ficus carica* L. varieties. *Asian Pac J Trop Biomed*. 2016;6(3):239–245. https://doi.org/10.1016/j.apjtb.2015.12.010
40. Ammar S, Contreras MdM, Belguith-Hadrich O, Segura-Carretero A, Bouaziz M. Assessment of the distribution of phenolic compounds and contribution to the antioxidant activity in Tunisian fig leaves, fruits, skins and pulps using mass spectrometry-based analysis. *Food Funct*. 2015;6(12): 3663–3677. https://doi.org/10.1039/C5FO00837A
41. Mahmoudi S, Khali M, Benkhaled A, et al. Fresh figs (*Ficus carica* L.): pomological characteristics, nutritional value, and phytochemical properties. *Eur J Hortic Sci*. 2018;83(2): 104–113. https://doi.org/10.17660/eJHS.2018/83.2.6 https://www.researchgate.net/publication/324808137_
42. Bachir-Bey M, Louaileche H. A comparative study of phytochemical profile and in vitro antioxidant activities of dark and light dried fig (*Ficus carica* L.) varieties. *Int J Phytopharm*. 2015;4(1):41–48. https://doi.org/10.31254/phyto.2015.4108
43. Soni N, Mehta S, Satpathy G, Gupta RK. Estimation of nutritional, phytochemical, antioxidant and antibacterial activity of dried fig (*Ficus carica*). *J Pharmacogn Phytochem*. 2014;3(2):158–165. https://www.phytojournal.com/vol3Issue2/Issue_jul_2014/3-3-1.1.pdf
44. Radwan S, Handal G, Rimawi F, Hanania M. Seasonal variations in antioxidant activity, total flavonoids content, total phenolic content, antimicrobial activity and some bioactive components of *Ficus carica* L. in Palestine. *Int J Pharm Tech Res*. 2020;13(4):329–340. https://doi.org/10.20902/IJPTR.2019.130404
45. Bachir Bey M, Richard G, Meziant L, Fauconnier ML, Louaileche H. Effects of sun-drying on physicochemical characteristics, phenolic composition and in vitro antioxidant activity of dark fig varieties. *J Food Process Preserv*. 2017;41(5):e13164. https://doi.org/10.1111/jfpp.13164
46. Puoci F, Iemma F, Spizzirri UG, et al. Antioxidant activity of a Mediterranean food product: “fig syrup”. *Nutrients*. 2011;3(3):317–329. https://doi.org/10.3390/nu3030317
47. Meziant L, Bachir-bey M, Bensouici C, Saci F, Boutiche M, Louaileche H. Assessment of inhibitory properties of flavonoid-rich fig (*Ficus carica* L.) peel extracts against tyrosinase, α-glucosidase, urease and cholinesterases enzymes, and relationship with antioxidant activity. *Eur J Integr Med*. 2021;43:101272. https://doi.org/10.1016/j.eujim.2020.101272
48. Zidi K, Kati DE, Benchikh Y, Bachir-Bey M, Ouandjeli D, Yahiaoui S. The use of modified atmosphere packaging as mean of bioactive compounds and antioxidant activities preservation of fresh figs (*Ficus carica* L.) from rare cultivars. *AUDJG Food Technol*. 2020;44(1):149–164. https://doi.org/10.35219/foodtechnology.2020.1.09
49. Viuda-Martos M, Barber X, Pérez-Álvarez JA, Fernández-López J. Assessment of chemical, physico-chemical, techno-functional and antioxidant properties of fig (*Ficus carica* L.) powder co-products. *Ind Crops Prod*. 2015;69:472–479.

https://doi.org/10.1016/j.indcrop.2015.03.005
50. Palmeira L, Pereira C, Dias MI, et al. Nutritional, chemical and bioactive profiles of different parts of a Portuguese common fig (*Ficus carica* L.) variety. *Int Food Res J*. 2019;126:108572. https://doi.org/10.1016/j.foodres.2019.108572
51. Saoudi M, El Feki A. Protective role of *Ficus carica* stem extract against hepatic oxidative damage induced by methanol in male Wistar rats. *Evid Based Complement Altern Med*. 2012;2012: 150458. https://doi.org/10.1155/2012/150458
52. Badgujar SB, Patel VV, Bandivdekar AH, Mahajan RT. Traditional uses, phytochemistry and pharmacology of *Ficus carica*: a review. *Pharm Biol*. 2014;52(11):1487–1503. https://doi.org/10.3109/13880209.2014.892515
53. Gullón B, Lú-Chau TA, Moreira MT, Lema JM, Eibes G. Rutin: a review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends Food Sci Technol*. 2017;67:220–235. https://doi.org/10.1016/j.tifs.2017.07.008
54. Yao LH, Jiang YM, Shi J, et al. Flavonoids in food and their health benefits. *Plant Foods Hum Nutr*. 2004;59(3):113–122. https://doi.org/10.1007/s11130-004-0049-7
55. Khan MK, Paniwnyk L, Hassan S. Polyphenols as natural antioxidants: sources, extraction and applications in food, cosmetics and drugs. *Plant Based Green Chem 2.0*. 2019: 197–235. https://doi.org/10.1007/978-981-13-3810-6_8
56. Comstock J, Gold MH. *Cosmeceuticals*. Taipei, China: The Leader Book Company Ltd;2021. https://doi.org/10.1159/isbn.978-3-318-06690-6
57. Baumann LS. *Cosmeceuticals and Cosmetic Ingredients*. Florida, USA: McGraw-Hill Medical;2015. ISBN: 978-0071793995
58. Chanda S, Baravalia Y, Kaneria M, Rakholiya K. Fruit and vegetable peels – strong natural source of antimicrobics. *Formatex Research Center*. 2010:444–450. https://www.researchgate.net/publication/261335157_
59. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med*. 1982;45(5):31–34. https://doi.org/10.1055/s-2007-971236
60. Wakawa HY, Fasihuddin BA. Brine shrimp lethality bioassay of *Abrus precatorius* (Linn) leaves and root extract. *Inter J Pharm Pharm Sci*. 2017;9(1):179–181. https://doi.org/10.22159/ijpps.2017v9i1.15057
61. Hamidi MR, Jovanova B, Panovska TK. Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. *Maced Pharm Bull*. 2014;60(1):9–18. https://doi.org/10.33320/maced.pharm.bull.2014.60.01.002
62. Naidu JR, Ismail R, Sasidharan S. Acute oral toxicity and brine shrimp lethality of methanol extract of *Mentha Spicata* L (Lamiaceae). *Trop J Pharm Res*. 2014;13(1):101–107. https://doi.org/10.4314/tjpr.v13i1.15
63. Meziant L, Boutiche M, Bachir bey M, Saci F, Louaileche H. Standardization of monomeric anthocyanins extraction from fig fruit peels (*Ficus carica* L.) using single factor methodology. *J Food Meas Charact*. 2018;12(4):2865–2873. https://doi.org/10.1007/s11694-018-9901-6
64. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J Agric Food Chem*. 1998;46(10):4113–4117. https://doi.org/10.1021/jf9801973
65. Jia ZS, Tang MC, Wu JM. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*. 1999;64(4):555–559. https://doi.org/10.1016/S0308-8146(98)00102-2
66. Kumaran A, Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *LWT Food Sci Technol*. 2007;40(2):344–352. https://doi.org/10.1016/j.lwt.2005.09.011
67. Giusti MM, Wrolstad RE. Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Curr Protoc Food Anal Chem*. 2001;1:F1.2.1–F1.2.13. https://doi.org/10.1002/0471142913.faf0102s00
68. Duman AD, Ozgen M, Dayisoylu KS, Erbil N, Durgac C. Antimicrobial activity of six pomegranate (*Punica granatum* L.) varieties and their relation to some of their pomological and phytonutrient characteristics. *Molecules*. 2009;14(5): 1808–1817. https://doi.org/10.3390/molecules14051808
69. Marco GJ. A rapid method for evaluation of antioxidants. *J Am Oil Chem Soc*. 1968;45(9):594–598. https://doi.org/10.1007/BF02668958
70. Öztürk M, Duru ME, Kivrak Ş, Mercan-Doğan N, Türkoglu A, Özler MA. In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three Agaricus species with fatty acid compositions and iron contents: a comparative study on the three most edible mushrooms. *Food Chem Toxicol*. 2011;49(6):1353–1360. https://doi.org/10.1016/j.fct.2011.03.019
71. Mansur JdS, Breder MNR, Mansur MCdA, Azulay RD. Determinaçäo do fator de proteçäo solar por espectrofotometria. *An Bras Dermatol*. 1986;61(3):121–124. https://pesquisa.bvsalud.org/portal/resource/pt/lil-34224
72. Sayre RM, Agin PP, LeVee GJ, Marlowe E. A comparison of in vivo and in vitro testing of sunscreening formulas. *Photochem Photobiol*. 1979;29(3):559–566. https://doi.org/10.1111/j.1751-1097.1979.tb07090.x
73. González-Minero FJ, Bravo-Díaz L. The use of plants in skin-care products, cosmetics and fragrances: past and present. *Cosmetics*. 2018;5(3):50. https://doi.org/10.3390/cosmetics5030050
74. Khan H, Akhtar N, Ali A. Effects of cream containing *Ficus carica* L. fruit extract on skin parameters: in vivo evaluation. *Indian J Pharm Sci*. 2014;76(6):560–564. https://www.researchgate.net/publication/270962825_
75. Ghimeray AK, Jung US, Lee HY, Kim YH, Ryu EK, Chang MS. In vitro antioxidant, collagenase inhibition, and in vivo anti-wrinkle effects of combined formulation containing *Punica granatum*, *Ginkgo biloba*, *Ficus carica*, and *Morus alba* fruits extract. *Clin Cosmet Investig Dermatol*. 2015;2015(8):389–396. https://doi.org/10.2147/CCID.S80906
76. Dini I, Laneri S. The new challenge of green cosmetics: natural food ingredients for cosmetic formulations. *Molecules*. 2021; 26(13):3921. https://doi.org/10.3390/molecules26133921
77. Mansuri R, Diwan A, Kumar H, Dangwal K, Yadav D. Potential of natural compounds as sunscreen agents. *Pharmacogn Rev*. 2021;15(29):47–56. https://doi.org/10.5530/phrev.2021.15.5
78. Maghsoudlou E, Kenari RE, Amiri ZR. Evaluation of antioxidant activity of fig (*Ficus carica*) pulp and skin extract and its application in enhancing oxidative stability of canola oil. *J Food Process Preserv*. 2017;41(4):e13077. https://doi.org/10.1111/jfpp.13077
79. Harzallah A, Bhouri AM, Amri Z, Soltana H, Hammami M. Phytochemical content and antioxidant activity of different fruit parts juices of three figs (*Ficus carica* L.) varieties grown in Tunisia. *Ind Crops Prod*. 2016;83:255–267. https://doi.org/10.1016/j.indcrop.2015.12.043
80. Gaaliche B, Ladhari A, Zarrelli A, Ben Mimoun M. Impact of foliar potassium fertilization on biochemical composition and antioxidant activity of fig (*Ficus carica* L.). *Sci Hortic*. 2019;253:111–119. https://doi.org/10.1016/j.scienta.2019.04.024
81. Veberic R, Colaric M, Stampar F. Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the northern Mediterranean region. *Food Chem*. 2008;106(1):153–157.

https://doi.org/10.1016/j.foodchem.2007.05.061

82. Elsner P, Maibach HI. *Cosmeceuticals and Active Cosmetics, Drugs versus Cosmetics*. Boca Raton, Florida, USA: CRC Press;2005. https://doi.org/10.1201/NOE0824759438
83. Jayaprakasha GK, Singh RP, Sakariah KK. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chem*. 2001;73(3):285–290. https://doi.org/10.1016/S0308-8146(00)00298-3
84. Rice-Evans C. 16-Screening of phenolics and flavonoids for antioxidant activity. *Antioxid food suppl in hum health*. 1999:239–253. https://doi.org/10.1016/B978-012543590-1/50017-2
85. Hassimotto NMA, Genovese MI, Lajolo FM. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J Agric Food Chem*. 2005;53(8):2928–2935. https://doi.org/10.1021/jf047894h
86. Yuan YV, Bone DE, Carrington MF. Antioxidant activity of dulse (Palmaria palmata) extract evaluated in vitro. *Food Chem*. 2005;91(3):485–494. https://doi.org/10.1016/j.foodchem.2004.04.039
87. Kim DO, Lee CY. HPLC Separation of Polyphenolics. *Curr Protoc Food Anal Chem*. 2002;6(1):I1.3.1–I1.3.16. https://doi.org/10.1002/0471142913.fai0103s06
88. de Oliveira-Júnior RG, Ferraz CAA, Souza GR, et al. Phytochemical analysis and evaluation of antioxidant and photoprotective activities of extracts from flowers of *Bromelia laciniosa* (Bromeliaceae). *Biotechnol Biotechnol Equip*. 2017; 31(3):600–605. https://doi.org/10.1080/13102818.2017.1288073
89. Dutra EA, Da Costa E Oliveira DA, Kedor-Hackmann ERM, Miritello Santoro MIR. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. *Rev Bras Cienc Farm*. 2004;40(3):381–385. https://doi.org/10.1590/S1516-93322004000300014
90. Ngoc LTN, Tran VV, Moon JY, Chae M, Park D, Lee YC. Recent trends of sunscreen cosmetic: an update review. *Cosmetics*. 2019;6(4):64. https://doi.org/10.3390/cosmetics6040064
91. Fardiyah Q, Ersam T, Slamet, Suprapto, Kurniawan F. New potential and characterization of Andrographis paniculata L. Ness plant extracts as photoprotective agent. *Arab J Chem*. 2020;13(12):8888–8897. https://doi.org/10.1016/j.arabjc.2020.10.015
92. Cefali LC, Ataide JA, Fernandes AR, et al. Evaluation of in vitro solar protection factor (SPF), antioxidant activity, and cell viability of mixed vegetable extracts from *Dirmophandra mollis* Benth, *Ginkgo biloba* L., *Ruta graveolens* L., and *Vitis vinífera* L. *Plants*. 2019;8(11):453. https://doi.org/10.3390/plants8110453
93. Lefahal M, Zaabat N, Ayad R, et al. In vitro assessment of total phenolic and flavonoid contents, antioxidant and photoprotective activities of crude methanolic extract of aerial parts of *Capnophyllum peregrinum* (L.) Lange (Apiaceae) growing in Algeria. *Medicines*. 2018;5(2):26. https://doi.org/10.3390/medicines5020026
94. Hu ST, Zhang XC, Chen F, Wang MF. Dietary polyphenols as photoprotective agents against UV radiation. *J Funct Foods*. 2017;30:108–118. https://doi.org/10.1016/j.jff.2017.01.009
95. Finney DJ. *Probit Analysis: A Statistical Treatment Of The Sigmoid Response Curve*. Cambridge, UK: Cambridge University Press; 1952. https://scholar.googleusercontent.com/scholar?q=cache:eifKoKp41VEJ:scholar.google.com/+Probit+analysis:+a+statistical+treatment+of+the+sigmoid+response+curve
96. Patil VV, Patil VR. Evaluation of anti-inflammatory activity of *Ficus carica* Linn. leaves. *Indian J Nat Prod Resour*. 2011; 2(2):151–155. http://hdl.handle.net/123456789/12134
97. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Biological screening of medicinal plants collected from Eastern Ghats of India using Artemia salina (brine shrimp test). *Int J Appl Sci Eng*. 2006;4(2):115–125. https://doi.org/10.6703/IJASE.2006.4(2).115
98. Rambaran TF, Ginigini J. Essential oil profiles of two *Rubus* varieties and the antimicrobial activities and lethality of their extracts. *Am J Essent Oils Nat Prod*. 2020;8(3):1–8. https://www.researchgate.net/profile/Theresa-Rambaran/publication/343240222_
99. Rufa L, Zhou YN, Wang R, Li JY. A study on the extract of Tartary buckwheat I. Toxicological safety of the extract of Tartary buckwheat. *Small*. 2001;21(8.4):204–208. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.602.5471&rep=rep1&type=pdf
100. Aligiannis N, Kalpoutzakis E, Mitaku S, Chinou IB. Composition and antimicrobial activity of the essential oils of two *Origanum* species. *J Agric Food Chem*. 2001;49(9):4168–4170. https://doi.org/10.1021/jf001494m
101. Oliveira AP, Valentão P, Pereira JA, Silva BM, Tavares F, Andrade PB. *Ficus carica* L.: metabolic and biological screening. *Food Chem Toxicol*. 2009;47(11):2841–2846. https://doi.org/10.1016/j.fct.2009.09.004
102. Jasmine R, Manikandan K, Brinda, et al. Evaluating the efficiency of *Ficus carica* fruits against a few drug resistant bacterial pathogens. *World J Pharm Pharm Sci*. 2014;3(2): 1394–1400. https://www.wjpps.com/wjpps_controller/abstract_id/829
103. Ashour A, El-Sharkawy S, Amer M, et al. Production of citric acid from corncobs with its biological evaluation. *J Cosmet Dermatol Sci Appl*. 2014;4(3):141–149. https://doi.org/10.4236/jcdsa.2014.43020
104. Lacombe A, Wu VCH, Tyler S, Edwards K. Antimicrobial action of the American cranberry constituents; phenolics, anthocyanins, and organic acids, against *Escherichia coli* O157: H7. *Int J Food Microbiol*. 2010;139(1–2):102–107. https://doi.org/10.1016/j.ijfoodmicro.2010.01.035
105. Puupponen-Pimiä R, Nohynek L, Alakomi HL, Oksman-Caldentey KM. Bioactive berry compounds – novel tools against human pathogens. *Appl Microbiol Biotechnol*. 2005;67(1):8–18. https://doi.org/10.1007/s00253-004-1817-x
106. Bilal M, Rasheed T, Iqbal HMN, Hu HB, Wang W, Zhang XH. Macromolecular agents with antimicrobial potentialities: a drive to combat antimicrobial resistance. *Int J Biol Macromol*. 2017;103:554–574. https://doi.org/10.1016/j.ijbiomac.2017.05.071
107. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. *Sci World J*. 2013;2013:162750. https://doi.org/10.1155/2013/162750
108. Xie YX, Yang WJ, Tang F, Chen XQ, Ren LC. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Curr Med Chem*. 2015;22(1):132–149. https://doi.org/10.2174/0929867321666140916113443
109. Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial activity of flavonoids and their structure – activity relationship: an update review. *Phytother Res*. 2019;33(1): 13–40. https://doi.org/10.1002/ptr.6208
110. Puupponen–Pimiä R, Nohynek L, Meier C, et al. Antimicrobial properties of phenolic compounds from berries. *J Appl Microbiol*. 2001;90(4):494–507. https://doi.org/10.1046/j.1365-2672.2001.01271.x
111. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr Res*. 2017;61:1361779. https://doi.org/10.1080/16546628.2017.1361779