Major Phytochemical Compounds, In Vitro Antioxidant, Antibacterial, and Antifungal Activities of Six Aqueous and Organic Extracts of *Crocus sativus* L. Flower Waste

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Abstract

**Purpose** After being considered as a neglected product, agricultural waste is nowadays considered of paramount importance. It has become a source of many chemical compounds with industrial, pharmaceutical, and food applications. This study aims to evaluate the primary phytochemical content, the antioxidant properties, and the antimicrobial activities of different extracts of saffron flower waste (SFE) against bacterial and fungal strains involved in diverse pathologies in southern Morocco.

**Methods** Total phenolic and flavonoid contents were determined. The antioxidant potentials were assessed by DPPH, FRAP, and β-carotene assays. The antimicrobial activity against four bacteria and four fungi was also evaluated. The findings in terms of the relationships between phytochemical content and all activities were depicted by PCA analyses.

**Results** SFEs contained large amounts of phenolic and flavonoid compounds that contribute to the significant antioxidant activities. Diethyl ether (DE), n-Butanol (n-B), and Ethyl acetate (EA) fractions respectively exhibited more DPPH scavenging capacity, FRAP reducing power, and β-carotene bleaching inhibition. DE and EA have a remarkable effect mainly against *Staphylococcus aureus* strain, compared to *Listeria monocytogenes*, *Escherichia coli* and *Klebsiella pneumoniae*. Both SFEs showed antifungal antagonism towards key fungi species involved in post-harvest mold and crop yield losses. *Botrytis cinerea* was more susceptible than *Fusarium solani*, *Penicillium expansum*, and *Penicillium digitatum*.

**Conclusion** These results reveal new data about extracts obtained from SFE that could be a potential source of natural antioxidant and antimicrobial agents opening new possibilities for their applications in the food system as a natural preservative and a sustainable alternative to conventional ingredients.
Graphical Abstract

Keywords Saffron flower waste · *Crocus sativus* L. · Antioxidant · DPPH · FRAP · β-Carotene · Antibacterial · Antifungal

Statement of Novelty

After pruning the stigmas used as commercial saffron, the rest of the flower, including the petals and stamens, are discarded as an agricultural waste. Each ton of final isolated stigmas produces about 350 tons of petals waste. Worldwide discarded waste is estimated at 146300 tons per year. This study proposes an additional valuation of the culture of saffron. Bioactive properties of saffron waste are suggested to be exploited for protective and curative applications. Their antimicrobial effects could also be used to control aggressive microorganisms, mainly against pathogenic bacteria isolated from public hospitals and against selected phytopathogenic fungi associated with significant crop losses. This method is particularly sought after as an alternative to conventional chemicals.

Introduction

Agricultural waste is the most abundant green, renewable, and sustainable resource residue [1]. Its valorization in most cases is advantageous both at the economic and environmental levels. The importance of this biomass as a renewable resource for biochemical has increased considerably during recent decades [2]. Nevertheless, unavoidable waste and byproducts can be considered a source of valuable nutrients, which can be used to solve resource limitations and health challenges of humankind in the twenty-first century [3]. Scientific evidence shows that these by products constitute an excellent alternative affording several bioactive compounds that can be isolated and reused. Due to their secondary metabolites, agricultural waste has become an actual product opening new perspectives for sustainable transitions in agriculture, healthcare, and food systems [4].

Medicinal and aromatic plants are rich in bioactive compounds, mainly polyphenols, vitamins, and enzymes that have specific properties allowing their use in the pharmaceutical, cosmetic, and food industries. These plants are widely grown, but their exploitation requires adapted solutions for biomass management. Among these, *Artemisia annua* [1], *Camellia sinensis* [5], *Ricinus communis* [6], *Mentha arvensis* [7], and *Thymus vulgaris* [8] constitute good examples. Their byproducts are the subjects of several studies focusing on extracting antioxidants, anti-inflammatory, and cellulosic materials used in cosmetic, medicinal, and polymer applications. However, several biomasses have not yet been exploited, such as saffron (*Crocus sativus* L.) flowers.
waste. This species has long been cultivated and used in herbal medicine as early as 1550 years before JC. Available pieces of information on saffron’s accurate spreading in the world are uncertain. Several authors trace back their origin to the Middle East, Central Asia, or particularly to the islands of southwestern Greece. Other studies suggest that it was selected and domesticated in Crete during the Bronze Age. Before being expanded to India, China, the countries of the Middle East and the Mediterranean basin, including Morocco, where it was probably introduced during the ninth century [9–12].

Saffron (*Crocus sativus* L.) is a sterile geophyte species with autumnal flowering belonging to the Iridaceae family, which reproduces exclusively in a vegetative pathway through corms. Its flowers are valuable for their red stigmas that are collected, dried, and used as a spice in food, as a dye, in perfumes and cosmetics preparation, and for medicinal purposes [13]. Saffron is also called “red gold” because of its very expensive, luxurious, and valuable spice across the globe [14, 15]. In addition, it is highly appreciated for its color (Crocin), fragrance (Picrocrocin), and flavor (Saffron) due to over 150 volatile and aromatic compounds [16, 17]. Morocco ranks fourth in terms of production after Iran, India, and Greece, with a production exceeding 10 t in 2020, according to the National Agency for the Development of Oasis Areas (ANDZOA). The traditional saffron production site is located in Taliouine and Taznakht in Taroudant Oasis Areas (ANDZOA). The traditional saffron production site is located in Taliouine and Taznakht in Taroudant and Ouarzazate cities, respectively. Moreover, the plant is reported to be adaptable to various environmental conditions. It grows well in arid and semi-arid areas as it can adapt to temperate and subtropical climates [18, 19].

In recent years, the demand for stigmas has increased exponentially, especially with the discovery of new therapeutic properties against cancer, depression, hypertension, and other health issues, including psychological problems, gaining more impetus, especially with the current health situation caused by the COVID-19 pandemic. This consumed part of the plant is rich in monoterpenoids, phytosterols, phenolic acids, flavonoids (all glycosidic derivatives of kaempferol), vitamins (riboflavin and thiamine), proteins, amino acids, minerals, starch, and gums [20]. Crocetin and crocin are the most important bioactive components for their effect on a wide range of therapeutic effects, mainly through vigorous antioxidant activity [21]. After pruning the stigmas, it is used as commercial saffron, and other parts of the flower, including the petals and stamen, are discarded as agricultural waste. One kg of stigmas produces about 350 kg of petals, equivalent to 150,000–200,000 flowers [22], which tend to increase parallelly with its production. The worldwide overall produced biomass is estimated at 146,300 t/y. Therefore, finding a solution to clear out this large volume of waste is essential. Saffron flower waste is a candidate for high-quality byproduct. Previous studies confirmed its richness in bioactive compounds, such as phenolics, flavonoids, glycosides, anthocyanins, and kaempferol. This fact could make of it a source of nutrients and phytochemicals with health benefits [23]. More studies focused on its antidepressant [24], antioxidant [25], antinociceptive, and anti-inflammatory activities [26]. This waste can also be used as a fertilizer for the soil, an ornament in dishes [26], or given to a limited extent to livestock as a food supplement during the wet and dry seasons [27]. Therefore, investing in this byproduct may be a promising approach to increase further economic value.

Meanwhile, the emergence of pathogenic bacterial strains poses a public health problem due to the consequences of these infections in terms of morbidity, mortality, and cost. In addition, pathogens and diseases are considered direct threats to food security [28]. *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and *Listeria monocytogenes* are frequently responsible for cutaneous, urinary, digestive, neonatal (including meningitis), nosocomial infections, and, in certain extreme cases, involved in infections that could be potentially fatal. Remaining among the most critical and worrying pathogens in human diseases, they are considered a case study for occupying an important place in nosocomial infectious pathology in southern Morocco [29]. In addition, the agri-food supply chain is also exposed to significant damages, mainly due to pathogenic fungi that usually infect the host from the soil after germination or through wounds sustained during planting, harvesting, or handling. Also, among the most frequent diseases that cause significant commercial losses in Morocco and worldwide *Penicillium expansum, Penicillium digitatum, Botrytis cinerea*, and *Fusarium solani* are associated with blue, green, grey post-harvest, and crown rot damages.

These damages are caused by oxidation, a chemical reaction necessary for the metabolism of living beings (energy production, cell growth) to cells and tissues and leads gradually to substantial dysfunctions involving downstream reactive oxygen species (ROS). Biological and synthetic antioxidants are added to overcome ROS, oxidative stresses, and ageing. It could prevent auto-oxidation and delay the shelf life of consumed products; however, these antioxidants are detrimental to long-term human use [30]. On the other hand, infectious diseases have been reduced, and the life spans of humans and livestock have increased due to antibiotics. Nevertheless, this has made many bacteria and fungi resistant to these conventional substances, resulting in potential threats to public health [31]. Accordingly, the search for new natural sources of antioxidants and antimicrobials has become an essential quest in the food, pharmaceutical, cosmetics, and polymer industries. Furthermore, they are enticing alternatives for several industries due to consumer demand for products of natural origin, affordable, inexpensive, low-risk natural resources, and resistance of pathogens to synthetic.
antibiotics that could be harmful to public health [32]. Therefore, this study aims to evaluate the primary phytochemical content, the antioxidant properties (DPPH, FRAP, and β-carotene), and the in vitro antimicrobial activities of different extracts of saffron flower waste against various bacterial and fungal strains involved in diverse pathologies.

Materials and Methods

Chemicals

The solvents Petrol ether and Diethyl ether were obtained from Honeywell Riedel–de Haen. Methanol, Tween 40, Potassium hexacyanoferrate, Trichloroacetic acid (TCA), Linoleic acid, and Sodium chloride were purchased from PanReac AppliChem. n-Butanol, Gallic acid, 2,2-Di-Phe-
nyl-1-Picryl-Hydrazyl (DPPH), Ascorbic acid, β-Caroten, Quercetin, Aluminum chloride AlCl₃, and Ciocalteu’s phenol reagent were all bought from SIGMA ALDRICH. Disodium hydrogen phosphate, Sodium dihydrogen phos-
phate, and Ferric chloride were acquired from Fisher Scien-
tific (S.A.). Ethanol was obtained from BIOSMART. Ethyl acetate and Dimethyl sulfoxide (DMSO) were purchased from LOBA Chemie. Sodium carbonate was acquired from HIMEDIA. The Muller-Hinton Agar and Potato Dextrose Agar mediums were purchased from Biokar Diagnostics (BK048 HA), France.

Preparation of Saffron Flower Extracts (SFEs)

The saffron flower waste (petals and stamens) was obtained in the morning from October–November 2020 from the Taznakht region in Morocco’s Anti-Atlas. After harvest and recovery of stigmas, the flowers were air-dried under shade at room temperature for several days, placed in the oven at 40 °C, crushed to the powder form, and stored in a dry, dark room temperature until use.

Saffron flower extraction was carried out using three methods. The first one is a decoction of the powder of the saffron flowers in distilled water for 1 hour at 100 °C. The second one is a maceration of the powder in Methanol for 48 h. While the third one is extraction in the Soxhlet apparatus and later with liquid–liquid extraction, it was carried out by hydro-ethanolic extraction using Soxhlet for 6 h. Finally, the extract obtained was evaporated on a rotary evaporator to remove the ethanol. The resulting aqueous suspension was partitioned sequentially with Petroleum ether, Diethyl ether, Ethyl acetate, and n-Butanol in a 1:1(v/v) ratio 3 times at room temperature. All extracts and the remaining water phase were filtered using a folded filter (Ø = 150 mm, porosity = 7–10 μm, Prat Dumas, France), then evaporated under reduced pressure using a rotary evaporator (Buchi R215).

The remaining solution was freeze-dried to obtain the corresponding Methanol, Water, Diethyl ether, Ethyl acetate, n-Butanol, and Aqueous fraction extracts. These extracts were kept at 4 °C in the dark until further analyses.

Determination of the Total Phenolic Content

The amount of total phenolic content (TPC) of saffron flower extracts was determined according to the Folin-Ciocalteu method modified by Singleton and Rossi [33]. First, the extract was transferred into a test tube (100 μl), and 0.5 mL of Folin-Ciocalteu agent (previously diluted tenfold with distilled water) was added and mixed for 1 min. Next, sodium carbonate (0.4 mL of 7% w/v) was added to the mixture and then placed in a water bath at 50 °C for 5 min. The absorbance was measured at 760 nm using a UV–Vis spectrophotometer (Thermo Fisher Scientific, Evolution ™ 300). The standard calibration curve was plotted using Gallic acid (r = 0.97). The total phenolic content was expressed as Gallic acid equivalents in milligram per gram of sample dry matter (mg GAE/g DM). All determinations were performed in triplicate.

Determination of Total Flavonoid Content

The total flavonoid content (TFC) was measured using aluminum chloride colorimetric assay with some modifications [34]. One mL of AlCl₃ (2%, w/v) solution was added to 1 mL of the test solution (standard or extract). The mixture has been vigorously shaken and then incubated at room temperature for 1 h. The absorbance of the mixture was identified at 430 nm. Quercetin was utilized as the reference compound for calculating the standard curve as it is widely found in plants. The total flavonoid content of samples is expressed in milligrams of Quercetin equivalent per gram of sample dry matter (mg QE/gDM). The analysis of all the samples and the standard were done in triplicate.

Evaluation of Antioxidant Activity

DPPH Radical Scavenging Activity

The radical scavenging activity of the extracts following the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was measured using the method of Sanchez-Moreno [35], modified as follows, 50 μl of the solutions of the extract at known concentrations were added to 1950 μl of methanolic solution DPPH (0.025 mg/mL of the Methanol).

The mixtures were shaken gently and incubated in the dark for 20 min. The scavenging activity corresponds to the absorbance of the samples due to the change in color (from deep violet to yellow) compared with the negative control containing 1950 μl of solution of DPPH and 50 μl of Methanol was
measured at 517 nm using a UV/visible spectrophotometer. Ascorbic acid was used as the positive control. The DPPH radical scavenging effect was calculated using the following equation:

\[
\text{DPPH} (%) = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100
\]

where \( \text{Abs}_{\text{control}} \) is the absorbance of the control and \( \text{Abs}_{\text{sample}} \) is the absorbance of the extract sample/standard at 20 min. The analysis of all the samples was done in triplicate. The antioxidant capacity of the extract was represented as \( \text{IC}_{50} \). The \( \text{IC}_{50} \) was measured from the pure compound’s antioxidant activities (%) used at various concentrations. A lower \( \text{IC}_{50} \) value shows the higher antioxidant activity of the plant extract.

**Ferric-Reducing Antioxidant Power (FRAP) Assay**

The principle of the FRAP method is based on the reduction of ferric ion (Fe\(^{3+}\)) to ferrous ion (Fe\(^{2+}\)) in the presence of antioxidants. According to Oyaizu (1986) procedure, the reducing power was carried out with some modifications [36]. In this essay, 0.5 mL of sample solution were mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium hexacyanoferrate 1% and then incubated to 50 °C in a water bath for 30 min. 1.25 mL of trichloroacetic acid (10%) was added to the solution, and the mixture was centrifuged at 2000 rpm for 10 min. In every 0.125 mL of the mixture, 0.625 mL of distilled water and 0.125 mL of FeCl\(_3\) 1% freshly prepared in distilled water were added. A blank without a sample is prepared under the same conditions. The absorbance of each sample solution was subsequently measured at 700 nm using a spectrophotometer. Ascorbic acid was used as the positive control. All measurements were done in triplicate.

**β-Carotene Bleaching Assay**

The assay was performed as described by Taga et al. with some modifications [37]. In brief, 0.5 mg of β-carotene dissolved in 1 mL of chloroform was mixed with 25 μl of linoleic acid and 200 mg of tween 40 in a flask. The chloroform was evaporated entirely in the Rotavapor at 40 °C, and 100 mL of distilled water was slowly added to the oily residue with vigorous agitation to form an emulsion. Then, 1 mL aliquot of the emulsion was added to a tube containing 140 μl of extract sample solution of different concentrations, and the absorbance was determined at 470 nm immediately against the blank solution consisting of the emulsion without β-carotene before and after heat treatment at 50 °C with regular time intervals of 20 min for 2 h.

\[
\text{Bleaching inhibition}(\%) = \left( \frac{A_{\text{β-carotene after 120 min of assay}}}{A_{\text{initial β-carotene}}} \right) \times 100
\]

With \( A_{\text{β-carotene after 120 min of assay}} \) is the absorbance value of β-carotene remaining in the samples 2 hours after the assay, and \( A_{\text{initial β-carotene}} \) is the absorbance value of β-carotene at the beginning of the experiment.

The concentration of extracts providing 50% inhibition (IC\(_{50}\)) was obtained by comparing the inhibition percentage of the curve to the extracts’ concentrations.

**In Vitro Antibacterial Activity**

**Bacterial Strains**

The antimicrobial activity of saffron flower extracts was evaluated against four human pathogenic bacteria species, two Gram-negative bacteria: *Escherichia coli* and *Klebsiella pneumoniae*, and two Gram-positive: *Staphylococcus aureus* and *Listeria monocytogenes CECT 4032*. The first three bacteria originated from the public hospital in Agadir, Morocco. These nosocomial bacteria were isolated at the Microbial Biotechnology and Vegetable Protection Laboratory (MBVPL) of the Faculty of Sciences, Ibn Zohr University, Agadir, Morocco.

**Well Diffusion Method**

The antibacterial activity was carried out based on the propagation method in the Mueller–Hinton agar medium [38]. The cups were made for each 6 mm diameter by scooping out medium with a sterilized cork borer from the Petri dishes containing the gel. They were then streaked with the bacterial suspension of turbidity equivalent to a standard of 0.5 McFarland, which corresponds to \( \approx 10^8 \) UFC/mL (O.D. = 0.08 to 0.1/λ = 625 nm) in 3 directions by sterile swab. Concentrations of 30, 100, and 300 mg/mL of extracts were prepared in DMSO (4% v/v) in 3 directions by sterile swab. Concentrations of 30, 100, and 300 mg/mL of extracts were prepared in DMSO (4% v/v) in 3 directions by sterile swab. Next, 40 μl of each dilution was added in cups, and Petri dishes were kept for 1 hour in the refrigerator at 4 °C to allow diffusion of the extracts into the medium. They were subsequently incubated at 37 °C for 24 h. DMSO (4% v/v) was used as a negative control. The zone of inhibition produced by each extract and dilution was measured in mm after 24 h of incubation. The test was repeated 3 times, and the means of results were reported.
Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

After confirming the antibacterial activity of all extracts, MIC and MBC were determined using a broth microdilution method against the test organisms [39]. Extracts were dissolved in DMSO (4% v/v) and then serially diluted in 96-well microtiter plates to obtain concentrations ranging from 3.125 to 100 mg/mL. Next, 100 μl of TSB (tryptic soy broth), 100 μl of extract, and 20 μl of an actively growing inoculum of the bacterial species were tested, adjusted to $10^6$ CFU/mL prepared in TSB, were added to each well. The medium sterility control (TSB alone) and the inoculum viability control (TSB with inoculum only) were tested. The cultures were incubated for 24 h at 37 °C. Microbial growth was determined based on the visual turbidity of the tubes. The lowest concentration that inhibits the bacterial strains’ development is the minimum inhibitory concentration (MIC).

To determine the minimum bactericidal concentration (MBC), 10 μl of suspension from each well showed no visible growth during MIC tests were placed in Petri dishes containing Muller and Hinton Agar culture medium and incubated at 37 °C for 24 h. After incubation, the lowest concentration, which did not show any growth (no colony) on the solid medium, was MBC.

In Vitro Antifungal Activity

Fungal Strains

Due to food contamination, spoilage caused by foodborne pathogens, and crop yield loss, four common pathogenic fungi in Morocco, including *Penicillium expansum*, *Penicillium digitatum*, *Botrytis cinerea* and *Fusarium solani* have been studied. These fungi responsible respectively, for blue, green, grey post-harvest and crown damage were isolated in the Microbial Biotechnology and Plant Protection Laboratory of the Faculty of Sciences, Ibn Zohr University, Agadir, Morocco.

In Vitro Effect on Mycelial Growth

A pure culture of these fungi was maintained on Potato Dextrose Agar (PDA) medium and stored at 4 °C. The pathogen inoculum of aqueous spore suspension was obtained from one-week-old culture plates incubated at 25 °C. Spores were recovered by pouring 10 mL of distilled water containing 0.05% (v/v) Tween 80 into the plates and filtering the suspension using two layers of sterile cheesecloth to remove hyphal fragments. The spore concentration was adjusted to $10^6$ spores per mL of sterile distilled water using a hemacytometer [40].

All extracts were screened for their antifungal effect using the disc-plate diffusion method. The spore suspension of the four fungi was deposited on Petri dishes containing PDA. 20 μl of extract at 30, 100, and 300 mg/mL concentration were deposited for each 6 mm disc. DMSO (4% v/v) was used as a negative control. The diameter of the zone of inhibition was measured in mm after 48 h of incubation. The test was performed in triplicates, and the means of the results were computed.

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

MIC of the extracts was determined by the agar dilution method. A series of concentrations of extracts 3.125, 6.25, 12.5, 25 and 50 mg/mL was carried out in test tubes containing melted PDA medium. After solidification of the medium, 7 μl of the arthrospore suspension (10^6 mL of arthrospores) were then deposited on the surface of the agar medium. The tubes were incubated at 25 °C for 48 h. The MIC value was taken for the lowest concentration of extract that allowed no visible growth of fungus. MFC was determined by reinoculating the contents of tubes that showed no visible mycelial growth on PDA medium for 48 h. Similarly, the MFC value was taken for the lowest concentration of the extract, which prevented the fungus development.

Statistical Analysis

Experimentations are done in triplicate. Results are given as mean ± standard variation. Obtained data are subjected to one-way Analysis of variance (ANOVA) with Tukey’s HSD test following the standard procedures at the 5% probability level. The data were analyzed using the statistical software CoStat software program (version 6.311, CoHort Software, Monterey, CA, USA). To expound major variations and co-variation for individuals, to interpret the underlying interrelationships between the phytochemical contents of the extracts and their antioxidant, antibacterial, and antifungal activities, Principal Component Analysis (PCA) and Pearson Correlation Coefficient analysis were considered and computed using ORIGIN pro 8 software and XLSTAT statistical computer software package (version 2018, Addinsoft Inc., New York, USA).

Results and Discussion

Solvent Extraction Yield

The methanolic, aqueous and hydro-ethanolic extract, which has been fractionated by partition with solvents of increasing
polarity: Ethyl acetate, Diethyl ether, and n-Butanol, have different extraction yields. The yield of the fractions relative to the weight of the dried saffron flower powder ranged from 1.22 to 27.87%. The extract with the highest yield was the Aqueous extract (66.3%), followed by the Methanolic extract (49.06%), the Aqueous fraction extract (27.87%), n-Butanol (10.97%), Diethyl ether (1.3%) and Ethyl acetate (1.22%).

**Total Phenolic and Flavonoids Contents**

The total polyphenol content of the different extracts of saffron flower obtained by different extraction methods was expressed in milligrams of gallic acid equivalent per gram of sample dry matter (mg GAE/g DM) using UV–Visible, and the equation of the calibration curve was $y = 0.0623x + 0.0296$, with $R^2 = 0.976$. The total flavonoid content was expressed in milligrams of Quercetin equivalent per gram of sample dry matter (mg QE/g DM) using the calibration curve equation: $y = 0.072x - 0.171$, with $R^2 = 0.999$.

The total phenolic content of the saffron flower extracts is shown in Fig. 1A. All extracts were rich in polyphenol. A very highly significant concentration of phenolic content was found in Diethyl ether (214.29 ± 12.68 mg of GAE/g DM) followed by n-Butanol (171.8 ± 13.41 mg of GAE/g DM), Ethyl acetate (154.32 ± 14.03 mg of GAE/g DM), Aqueous fraction (130.39 ± 13.79 mg of GAE/g DM), Methanol (114.66 ± 3.48 mg of GAE/g DM) and Water (104.82 ± 4.36 mg of GAE/g DM).

The total flavonoid content of the saffron flower extracts is shown in Fig. 1B. The extracts had the same ranking as the phenolic results, with very highly significant dominance for Diethyl ether (84.46 mg of QE/g DM ± 16.10) followed by n-Butanol (60.59 mg of QE/g DM ± 11.38), Ethyl acetate (46.88 mg of QE/g DM ± 4.80), Aqueous fraction (39.06 mg of QE/g DM ± 10.85), Methanol (31.27 mg of QE/g DM ± 0.73) and Water (26.88 mg of QE/g DM ± 2.60).

The presented results are higher than saffron cultivated in different areas produced in the northwest Italian Alps (TPC ranging between 888.35 and 3642.95 mg GAE/100 g DW) [41], in Lebanon (160 mg GAE/100 g DW) [42], in Iran (1.96 ± 0.12 g GAE/100 g DW) [43], in Algeria (TPC = 69.187 mg GAE/g DM and TFC = 4.322 mg CE/g DM) [44], in Morocco (TPC of different parts of saffron varies from 37 ± 4 to 94 ± 17 mg of GAE/g and TFC varies from 27 ± 4 to 327 ± 61 mg of Q/g) [45]. These authors have found variable values of phenolic and flavonoid compounds due to different origins, altitudes, growing conditions, and picking periods. In addition, other parameters can also be considered as possible causes of the variations of these compounds, such as geographical conditions, genetic factors, temperatures, storage, and sample preparation techniques in
the laboratory [46]. These results indicate that saffron flower waste is an accessible source of natural phenolic and flavonoid compounds. Lachguer et al. confirm that the saffron flower contains phenolic and flavonoid components using FTIR spectroscopy [47]. Kaempferols and anthocyanins are the main compounds of the high-phenolic content of the saffron flower [48].

**Antioxidant Activity**

The application of several complementary tests has been the most appropriate approach to identifying the antioxidant activity of a plant extract [49]. In the present study, three biochemical assays were used to determine the antioxidant potential of saffron flowers. Each assay represented the antioxidant activity of the test at different point of view. The obtained results of IC$_{50}$ are summarized in Table 1.

**DPPH Radical Scavenging Activity**

Antioxidant properties, mainly radical scavenging activities, are of great interest due to free radicals’ harmful repercussions for food and biological systems. The dominance of free radicals in food accelerates lipid oxidation, degrades food quality, and reduces consumer demand [50]. In general, DPPH is used to evaluate antioxidants’ free radical scavenging activity. Plant extracts’ antioxidant effect on DPPH radical scavenging may be due to their hydrogen donating ability, which reduces the stable purple DPPH free radical to yellow non-radical form DPPH-H [51].

Results corresponding to incubation of DPPH with different concentrations of SFE and with ascorbic acid are shown in Fig. 2A. As shown in this Figure, the DPPH scavenging capacities of the six extracts increased with increases in their concentrations, corresponding to the decrease in absorbance at 517 nm induced by compounds with an antioxidant effect. Ascorbic acid presented the highest scavenging ability. All the extracts tested showed a concentration-dependent relationship. At a 1 mg/mL concentration, the radical scavenging capacities of ascorbic acid, Diethyl ether, Ethyl acetate, n-Butanol, Aqueous fraction, Water and Methanol approached 95.944, 75.53, 62.58, 55.84, 44.58 and 38.75% respectively. Diethyl ether had the highest antioxidant potential (IC$_{50}$ = 309.44 μg/mL), followed by Ethyl acetate (IC$_{50}$ = 721.09 μg/mL), n-Butanol (IC$_{50}$ = 883.96 μg/mL), Aqueous fraction (IC$_{50}$ = 888.77 μg/mL). Water and Methanol extracts present the weakest antioxidant activity, with 1226.98 and 1364.8 μg/mL IC$_{50}$ values, respectively.

**Ferric-Reducing Antioxidant Power (FRAP) Assay**

The reducing power is evaluated by reducing Fe$^{3+}$ to Fe$^{2+}$ by electron transfer from an antioxidant. The high absorbance of the reaction mixture indicates high reducing power and its ability to behave as an antioxidant by donating electrons [52]. The results of antioxidant screening using the reduction method are depicted in Fig. 2B. The reducing capacities of SFEs were measured and compared with ascorbic acid. A dose-dependent relationship was observed in all of the extracts. The reducing absorbance of Diethyl ether, Ethyl acetate, n-Butanol, Aqueous fraction, Methanol, and Water

| Extracts          | IC$_{50}$ (μg/mL) | FRAP         | β-Carotene   |
|-------------------|-------------------|--------------|--------------|
|                   | DPPH              |              |              |
| Aqueous           | 1226.98 ± 52.86$^a$| 56.59 ± 5.22$^{***}$ | 398.84 ± 10.11$^{***}$ |
| Aqueous fraction  | 888.77 ± 87.36$^b$| 22.74 ± 0.97$^{cd}$ | 26.74 ± 1.43$^d$ |
| Diethyl ether     | 309.44 ± 33.30$^c$| 19.82 ± 3.75$^{d}$ | 309.41 ± 11.65$^b$ |
| Ethyl acetate     | 721.09 ± 29.96$^b$| 32.60 ± 6.46$^{bc}$ | 91.07 ± 10.71$^c$ |
| Methanol          | 1364.80 ± 95.62$^{****}$ | 40.57 ± 3.46$^{b}$ | 47.86 ± 11.06$^{d}$ |
| n-Butanol         | 883.96 ± 152.60$^b$| 29.55 ± 2.50$^{cd}$ | 89.57 ± 8.09$^c$ |
| Ascorbic acid     | 2.07 ± 0.04$^{d}$  | 1.18 ± 0.00$^{c}$ | -            |
| Quercetin         | 1.51 ± 0.10$^{d}$  | 1.11 ± 0.01$^{s}$ | 1.58 ± 0.02$^{s}$ |
| Tukey’s test      | F = 146.12        | F = 83.42     | F = 99.151   |
| (α = 0.05)        | (Df = 7)          | (Df = 6)     |              |
|                   | ***F < 0.001      | ***P < 0.001  | ***P < 0.001 |

Different letters are used for significant differences in antioxidant activities between saffron extracts.***P < 0.001

The values mentioned in bold in tables 1 correspond, respectively, to the maximum values found for IC$_{50}$ of each method for evaluating antioxidant activity, the maximum zone of inhibition for each bacterium and the maximum zone of inhibition for each fungus.
were 2.30, 1.69, 1.54, 1.54, 1.50, and 1.03 at 0.125 mg/mL, respectively. Diethyl ether had the highest reducing potential (IC\textsubscript{50} = 19.82 ug/mL) then, Aqueous fraction (IC\textsubscript{50} = 22.74 ug/mL), n-Butanol (IC\textsubscript{50} = 29.55 ug/mL), Ethyl acetate (IC\textsubscript{50} = 32.6 ug/mL), Methanol (IC\textsubscript{50} = 40.57 ug/mL), Water (IC\textsubscript{50} = 56.59 ug/mL). They were less efficient than ascorbic acid (IC\textsubscript{50} = 1.18 ug/mL). Therefore, it can be said that all SFEs can stop the radical chain reaction by creating the reactions between transferred electrons and free radicals and subsequently converting them into more stable products.

**β-Carotene Bleaching Assay**

The antioxidant capacity of SFEs was also determined using β-carotene bleaching assay. In this test, the oxidation of the linoleic acid areas was attacked by the β-carotene radical, which results in a decrease in absorbance at 470 nm and the disappearance of its yellow color.

β-carotene bleaching inhibition is shown in Fig. 2C. The interaction of antioxidant potency with β-carotene depended on the concentrations of the extracts. The antioxidant power decreased in the following order Ethyl acetate > Aqueous fraction > Diethyl ether > Methanol > n-Butanol > Water in the concentration of 1 mg/mL of SFEs. The bleaching kinetics of β-carotene correspond to the decrease in absorbance of β-carotene in the presence and absence of different fractions of saffron flower (Fig. 2D). The control sample without adding the antioxidant oxidized most rapidly, followed by water and other extracts. The β-carotene bleaching test in the absence of an antioxidant is based on a rapid discoloration of β-carotene translated by a loss of its color due to its oxidation. However, the presence of an antioxidant prevents this oxidation by neutralizing the free radicals formed from linoleic acid [53]. Therefore, the absorbance and color are kept for a more extended period. This work confirms that all saffron flower extracts have antioxidant power and prevent β-carotene bleaching. This potency may be due to the phenolic and flavonoid compounds of SFEs.

The FRAP test assesses the ability of the antioxidant to transfer an electron (Single Electron Transfer test SET). By contrast, the β-carotene bleaching test shows the antioxidant capacity to transfer a proton (Hydrogen Atom transfer test HAT), whereas the DPPH test is both a SET and HAT test [54]. This difference in the results obtained for each method is due to each method’s different specificity and sensitivity [55].

These results show that the six extracts of saffron flower waste revealed a solid scavenging effect, an excellent reducing power, and prevention of the bleaching of β-carotene and, therefore, a high antioxidant activity.
These results are consistent, superior, or inferior to those reported by other authors. Several in vitro antioxidant methods have been assayed on saffron byproducts; Azghandi et al. reported the antioxidant capacity of polyphenol, flavonoids, and carotenoid contents of methanolic extract in various parts of saffron. The IC$_{50}$ of the DPPH method for stigma, style, stamen, petal, and corm was 4.94, 123.68, 58.97, 46.02, and 720.49 μg/mL, which are lower than in our results. The stigma has the highest, and the corm has the lowest DPPH and FRAP methods [43]. Sánchez-Vioque et al. reported that petals and leaves constitute a potentially rich source of antioxidants and metal chelating compounds. Best antioxidant properties were observed for leaf extract, where as petal extract showed an extensive inhibition of β-carotene oxidation and significant scavenging NO radical and Cu$^{2+}$ chelating activities [22]. Carlo I.G. Tuberoso et al. noticed that Juices obtained from cold-pressed saffron floral byproducts 48 h after the harvest had the highest antioxidant activity using FRAP and DPPH methods [56]. Caser et al. showed that fresh saffron tepals are a source of nutraceutical components, with antioxidant activity determined by ABTS, DPPH, and FRAP differing depending on solvent and extraction method [41]. Also, Stelluti et al. observed that all assays evaluated, namely, FRAP, ABTS, and DPPH, revealed the presence of antioxidant activity in aqueous and methanolic extracts [57]. This antioxidant activity varies according to different factors, particularly the conditions of preparation of the extracts, the type of extraction, the solvent used, and other factors related to the plant. The antioxidant properties of SFE are related to bioactive components such as polyphenols and flavonoids, as assessed by the PCA analysis (Fig. 3), as exhibited by Diethyl Ether extract, n-Butanol, and Ethyl acetate. Other active compounds concerned mainly anthocyanins, hydrolyzable, and condensed tannins [44]. Kaempferol and delphinidin isolated in saffron flower have an important antioxidant capacity, and these can be applied in foods, phytopharmaceuticals, and cosmetics.

**Antibacterial Examination**

The antibacterial activity of six extracts was tested against two Gram-negative strains of *Escherichia coli* and *Klebsiella pneumoniae* and two Gram-positive strains of *Staphylococcus aureus* and *Listeria monocytogenes*. In vitro antibacterial potential of different extracts was detected by the presence or absence of inhibition zone observed around the wells. Among the saffron flower extracts tested for antibacterial activity, only the two Diethyl ether and Ethyl acetate fractions revealed the presence of an inhibition zone (Table 2). Results show that the inhibitory effect increased as the concentration of extracts increased. Diethyl ether showed the highest inhibition zone diameter against *Staphylococcus aureus* with 16.13 mm at 300 mg/mL. In contrast, Ethyl acetate showed a lower inhibition zone against *Klebsiella pneumonia* with 8.13 mm of diameter in the concentration of 30 mg/mL. Diethyl ether has more antibacterial power.

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**Fig. 3** PCA of the correlations between phytochemical content (phenolics and flavonoids), DPPH radical-scavenging activity, ferric reducing antioxidant power (FRAP) and β-carotene bleaching essay of six different saffron flower extracts (SFEs)
Table 2  Bacterial inhibition zones obtained by different saffron flower extracts applied against four strains (diameter in millimeters ± standard deviation)

| SFEs  | Doses (mg/mL) | Bacterial strains tested in vitro by well diffusion assay (mm) | Strains Tukey Test (α = 0.05) |
|-------|---------------|---------------------------------------------------------------|-------------------------------|
|       |               | Escherichia coli     | Klebsiella pneumoniae | Staphylococcus aureus | Listeria monocytogenes |
|       |               | Gram-negative        | Gram-positive         |                      |                          |
|       |               |                      |                      |                      |                          |
| DE    | 30            | 11.27 ± 2.19<sup>Aa</sup> | 12.88 ± 0.70<sup>abA</sup> | 12.01 ± 2.97<sup>abA</sup> | 11.05 ± 0.57<sup>abA</sup> | F = 0.56 | Df = 3 | (ns) | P = 0.655 |
|       | 100           | 12.79 ± 0.48<sup>abA</sup> | 13.08 ± 2.61<sup>abA</sup> | 14.80 ± 1.41<sup>abA</sup> | 13.05 ± 0.70<sup>abA</sup> | F = 1.06 | Df = 3 | (ns) | P = 0.416 |
|       | 300           | 13.96 ± 1.82<sup>abA</sup> | **15.15 ± 1.03<sup>ab**</sup>**<sup>abA</sup>** | **16.13 ± 2.11<sup>ab(ab)</sup>**<sup>abA</sup> | **15.35 ± 1.81<sup>ab(ab)</sup>**<sup>abA</sup> | F = 0.79 | Df = 3 | (ns) | P = 0.529 |
| EA    | 30            | 12.87 ± 0.55<sup>aA</sup> | 8.13 ± 1.07<sup>bB</sup> | 11.83 ± 1.71<sup>aA</sup> | 10.40 ± 1.80<sup>bAB</sup> | F = 6.62 | Df = 3 | (ns) | P = 0.015 |
|       | 100           | **14.28 ± 1.09<sup>ab(ab)</sup>**<sup>aA</sup> | 9.45 ± 0.55<sup>bB</sup> | 11.26 ± 1.08<sup>abB</sup> | 11.09 ± 0.60<sup>bB</sup> | F = 16.01 | Df = 3 | (ns) | P < 0.001 |
|       | 300           | 13.50 ± 0.50<sup>aA</sup> | 12.67 ± 1.74<sup>abA</sup> | 13.50 ± 0.98<sup>aA</sup> | 12.88 ± 0.70<sup>abA</sup> | F = 0.28 | Df = 3 | (ns) | P = 0.840 |

SFEs  saffron flower extracts, DE diethyl ether extract, EA Ethyl ether extract, lower- and upper-case letters: significant differences among saffron flower extract doses and among bacterial strains, respectively.*P < 0.05, **P < 0.01, ***P < 0.001, ns not significant

The values mentioned in bold in tables 2 correspond, respectively, to the maximum values found for IC50 of each method for evaluating antioxidant activity, the maximum zone of inhibition for each bacterium and the maximum zone of inhibition for each fungus.

![Fig. 4](image.png)  PCA of the effects of two active saffron flower extracts against four bacterial strains. Diethyl ether and Ethyl acetate extracts were considered at three concentrations, 30, 100, and 300 mg/mL. Staphylococcus aureus, species causing skin infections, were most susceptible to SFE at different concentrations than Escherichia coli, Listeria monocytogenes, and Klebsiella pneumoniae.
than Ethyl acetate for Klebsiella pneumonieae and Listeria monocytogenes. This difference is not significant for the other bacteria. In general, gram-positive bacteria have more abilities than gram-negative bacteria. The latter provides a much more complex barrier system preventing the permeation of substances with an antimicrobial effect. This is due to the cell wall’s specialized structure and the outer envelope’s presence, resulting in the impermeability of these microbes to these substances [58]. Therefore, this study showed that the inhibitory effect of different extracts of saffron flower on Gram-positive bacteria was higher than on Gram-negative bacteria. Statistically, this difference is not significant except for Ethyl acetate extracts that had an antibacterial influence against Escherichia coli more than other bacteria for 30 and 100 mg/mL concentrations. The PCA analysis of the effect of Diethyl ether and Ethyl acetate confirms these findings (Fig. 4). Both extracts under investigation exhibited concentration-dependent antimicrobial activity against Gram-positive and Gram-negative bacteria. Staphylococcus aureus species causing skin infections was the most sensitive compared to other bacteria, with remarkable sensitivity of Escherichia coli to ethyl acetate extract. These antimicrobial activities may be due to the content of saffron flowers in polyphenols, flavonoids, terpenes, and fatty acids [59]. The mechanism of this activity is related to the phenolic compounds that attack cell walls (ability to complex with extracellular and soluble protein), the membranes acting on their permeability, and the release of intracellular constituents. Other membrane functions are also involved in electron transport, enzymatic activity, or nutrient uptake [60].

MIC and MBC values of saffron flower extracts against the tested bacteria are displayed in Table 3. If the ratio MBC/MIC ≤ 4, the effect is defined as bactericidal, and if the ratio is > 4, the effect is considered as bacteriostatic [61]. The MBC/MIC ratios are displayed in Table 3. Both extracts showed antimicrobial activity against all four bacteria. The most active extract was the Diethyl ether with lower MIC and MBC (12.5 and 25 mg/mL, respectively, against Staphylococcus aureus). MIC ranged between 12.5 and 50 mg/mL, while MBC was around 25 and > 100 mg/mL. Since MBC/MIC ≤ 4, both extracts are considered as bactericide agents. The most promising activity concern the gram-positive bacteria Staphylococcus aureus, followed by other bacteria. Gandomi Nasrabad et al. revealed that ethanolic, methanolic, and aqueous extracts of saffron petals have more antimicrobial effects against Staphylococcus aureus than Escherichia coli, and MIC of all extracts was estimated at 40 mg/mL which were higher than the values of MIC found in this work [62]. Also, A. J. Sales and M. Pashazadeh showed that the methanolic extract of the saffron petal has an inhibitory effect on the Gram-positive more than Gram-negative expressed as MIC and MBC in the range of 6.25–100 and 12.5–200 mg/mL, respectively [63]. Shadmehri et al. reported that the MIC and MBC of extracts of petals were lower than the results of the present study, with a range of 62.5–250 and 250–500 ug/mL, respectively [64]. Among the main reasons for the difference in results in different studies are the differences in the composition of the saffron flower, the extraction conditions and the solvent used, the sensitivity of the bacteria to the different extracts, and the methods of evaluating the antibacterial properties. Likewise, Bahman Fazeli-Nasab showed that the hydroalcoholic extract of saffron petals was effective on all the bacterial strains tested. The bacterial strain that exhibits the largest zone of inhibition is Listeria monocytogenes, followed by Escherichia coli [65]. Gram-positive bacteria are usually more sensitive to plant-origin antimicrobials than Gram-negative bacteria that are more resistant. In our study, Ethyl acetate extract is more inhibitory and bactericidal to Escherichia coli than Listeria monocytogenes. Compared to Gram-positive bacteria, the sensitivity of these Gram-negative bacteria may be due to cell wall structure, physiology, and degree of bacterial cell contact, which can block the penetration of the active compounds embodied in the extracts. Another study carried out on Crocus sativus petals reported an inhibitory effect on Escherichia coli more than Staphylococcus aureus [64]. Asgarpanah et al. show that the different extracts of saffron petals and stamens have antibacterial activity on S. aureus, S.enteric, S. dysenteriae, B. cereus, E. coli, and Shigella dysenteriae while Gram-negative bacteria were the most sensitive [66].

| Bacterial strains | SFE concentration (mg/mL) | Diethyl ether | Ethyl acetate |
|------------------|---------------------------|---------------|---------------|
|                  | MIC | MBC | MBC/MIC | MIC | MBC | MBC/MIC |
| Escherichia coli | 25  | 50  | 2       | 25  | 50  | 2       |
| Klebsiella pneumoniae | 25  | 50  | 2       | 25  | 50  | 2       |
| Staphylococcus aureus | 12.5 | 25  | 2       | 25  | 50  | 2       |
| Listeria monocytogenes | 25  | 50  | 2       | 50  | >100 | >2      |
Zara et al. reported no differences between Gram-positive and Gram-negative bacteria tested for saffron stamen [59]. Gahruie et al. reported that *Klebsiella pneumoniae* is the most resistant bacteria compared to *Staphylococcus aureus* and *Salmonella typhimurium* [67]. In general, the results of this work confirm that saffron flower waste extract has remarkable antibacterial activity against the tested bacteria. Therefore, it can be a suitable substitute for conventional chemical drugs in treating infections under the herbal medicine category. In vivo clinical trials are recommended to prove these results.

**Antifungal Examination**

The Antifungal activity of the six saffron flower extracts was also evaluated against four different key fungi species. These species belong to different taxa and cause post-harvest damages and crop yield losses: blue, green, grey molds, and crown rot. Tested fungi strains for these diseases were *Penicillium expansum*, *Penicillium digitatum*, *Botrytis cinerea*, and *Fusarium solani*.

Similarly, Diethyl ether and Ethyl acetate showed outstanding activities. The diameters of zone inhibition obtained by the disc diffusion method reported in Table 4 show that the exhibited diameters ranged from 7.73 to 17.57 mm depending on the extract, the concentrations, and fungi strains. At large, diameters were significantly limited for Ethyl acetate compared to Diethyl ether extract for the tested strains except for *Fusarim solani*. *Botrytis cinerea* showed no significant differences between both extracts. This latter species is responsible for grey mold disease. It was significantly more susceptible than the other fungi species. PCA analysis of the effect of Diethyl ether and Ethyl acetate extracts at different concentrations on *Botrytis cinerea* is presented in Fig. 5. Accordingly, the PCA projection presents *Fusarium solani* more susceptible to Ethyl acetate. In contrast, *Penicillium expansum* and *Penicillium*...
digitatum were clustered separately for exhibiting mainly similar susceptibility to Diethyl ether extract.

MIC and MBC values of saffron flower extracts against the tested fungi presented in Table 5 showed that Diethyl ether extract had a high fungicidal potential (MIC = 6.25 mg/mL and MFC = 6.25 mg/mL mainly against Botrytis cinerea and Fusarium solani). MIC ranged between 6.25 and 50 mg/mL, while MBC ranged between 6.25 and > 50 mg/mL. Similarly, MFC/MIC ≤ 4. Therefore, both extracts are considered fungicide agents rather than having a biostatic effect. Previous works explained the effect of plant extracts on fungal pathogens by the contents of secondary metabolites such as phenolic compounds, flavonoids, and terpenoids that cause deterioration of fungal hyphae and perturbation of mycelial growth [68]. Plant extracts induce either cell wall and/or plasma membrane disruptions, which appear
to cause cell wall alterations and damage. In this study, both extracts, Diethyl ether and Acetate ethyl with antifungal power, are apolar with lipophilic properties. These substances interfere with the cell wall synthesis reactions, cause significant changes in the morphology of the pathogen and lead to cell lysis [69]. Chen et al. confirmed the effect of saffron petals and stamens on certain fungi like Botrytis cinerea and Penicillium italicum [70]. To our knowledge, no publications concerned other fungi strains. The results obtained indicate that SFEs have potentialities to be considered for developing adapted drugs to treat diseases caused by microbes. In vitro testing of plant extracts is a first step in selecting extracts with antifungal activities. However, other in vivo tests are needed to confirm these results.

Fig. 5 Geometric PCA of the impacts of two active saffron flower extracts against four fungal strains. Diethyl ether and Ethyl acetate extracts were considered at three different levels, 30, 100, and 300 mg/mL. Botrytis cinerea strain, the causal agent of post-harvest grey mold fruit damage is more susceptible to both SFEs at different levels than Fusarium solani, Penicillium digitatum, and Penicillium expansum.

Table 5 MIC and MFC values obtained for active saffron flower waste extracts (mg/mL)

| Fungal strains      | SFE concentration (mg/mL) |       |       |       |
|---------------------|----------------------------|-------|-------|-------|
|                     | Diethyl ether              | Ethyl acetate |
|                     | MIC | MFC | MFC/MIC | MIC | MFC | MFC/MIC |
| Penicillium expansum| 25  | 25  | 1       | 50  | > 50 | > 1     |
| Penicillium digitatum| 12.5 | 12.5 | 1       | 25  | > 50 | > 2     |
| Fusarium solani     | 6.25 | 6.25 | 1       | 25  | 25  | 1       |
| Botrytis cinerea    | 6.25 | 6.25 | 1       | 12.5 | 50  | 2       |
Conclusion

This research compared the quality of several saffron flower extracts, supplementing available data on this valuable crop. The present study demonstrated that extraction solvents for saffron flower waste affected extraction yield, phenolics, flavonoid contents, antioxidant, antibacterial, and antifungal activities. Diethyl ether, n-Butanol, and Ethyl acetate extracts were richer in terms of phenols and flavonoids major compounds. These phytochemical contents correlated with DPPH scavenging capacity, FRAP reducing power, and β-carotene bleaching inhibition activities. Diethyl ether and Ethyl acetate extracts were the only two extracts exhibiting microbial activities against Staphylococcus aureus that causes virulent human diseases, and Botrytis cinerea, the fungal species responsible for enormous grey mold crop losses. These activities are suspected to be due to specific drivers within the SFEs phytochemical patterns.

This multifaceted study revealed that saffron flower extracts might be potential natural antioxidant and optimal antimicrobial agent sources. Therefore, the saffron flower “waste” could be used to provide valuable bioactive compounds, potentially useful for various stored products, nosocomial and crop-borne pathogens. As a result, it should find a place in several applications in the food system, such as preservatives, dyes, antioxidants, enrichment additives, and many others. These will contribute to implementing the zero-waste concept, an effective strategy promoting significant recovery of agro-industrial waste generated in value-added products.

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Data Availability Data is available on reasonable request.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Consent to Participate All authors agree to participate in the current work.

Consent for Publication All authors agree to publish the findings of the current research.

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