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Antioxidant and antimicrobial activities of ginger, saffron and red pepper extracts

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ABSTRACT

The current study investigated the antioxidant and antimicrobial activities of ginger, saffron, and red pepper extracts. The extracts of plants were prepared by adding plant powder to distilled water and stirring at 60°C for 24 h. The antioxidant capacity was measured utilizing DPPH radical scavenging. The increasing order of antioxidant activity at 1 mg/mL concentration was as follows: saffron (78.5%) < ginger (79.3%) < red pepper (83.4%) < ascorbic acid (99.7%, as control). The TPC for ginger, red pepper, and saffron was 9.78 ± 1.42, 4.72 ± 0.85, and 6.41 ± 0.63 mg GAE/g, respectively. The TFC for ginger, red pepper, and saffron was 3.74 ± 0.65, 8.78 ± 0.42, and 2.61 ± 0.34 mg QE/g, respectively. The antimicrobial activities of plant extracts were assessed using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The saffron extract had the highest inhibitory effect against *Bacillus cereus* (MIC = 3.12 mg/mL, MBC = 6.25 mg/mL) and *Staphylococcus aureus* (MIC = 6.25 mg/mL, MBC = 12.5 mg/mL). Ginger extract also showed high efficacy, especially against *S. aureus* (MIC = 3.12 mg/mL). In contrast, red pepper extract had a less potent effect on the studied species. The findings of this study showed that plant extracts had suitable antioxidant and antimicrobial properties, making them a good choice for use as natural preservatives in various industries.

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1. Introduction

Recently, growing concern regarding synthetic additives and antibiotic resistance has triggered an increasing interest in natural alternatives, particularly plant-derived compounds.

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Herbs with medicinal and culinary properties have received substantial attention thanks to their antimicrobial and antioxidant activities, as well as their affordability. Specifically, extracts of plants are being studied as natural food additives for their potential to inhibit foodborne pathogens and retard spoilage, providing a promising strategy for improving food safety and prolonging food shelf life. Among them, ginger (*Zingiber officinale* Rosc.), red pepper (*Capsicum annuum* L.), and saffron (*Crocus sativus* L.) have been



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widely employed as spices in foods for a long time because of their unique color, taste, and aroma (1-5).

Ginger is an erect perennial plant that is commonly grown in tropical areas, including West Africa, India, Jamaica, and China. The fresh ginger root has been historically prescribed for colds, nausea, coughs, heart palpitations, inflammation, gastrointestinal disorders, appetite loss, and arthritic pain. The bioactive ingredients of ginger, specifically gingerols, shogaols, and zingerone, have antioxidant, anti-inflammatory, and anticancer activities. Furthermore, this plant contains antimicrobial agents such as eugenol, thymol, 1,8-cineole, pinenes, linalool, and terpineol, which are effective against a variety of microorganisms (6, 7). Ginger is generally considered safe, but overdosing can result in gastrointestinal irritation in specific individuals (8).

Peppers are globally popular due to their flavor, vibrant color, and nutritional benefits. The color of peppers depends on various factors, including the levels of specific compounds, ripening changes, genotypes, and the time of year when they are cultivated. The red color results from capsaanthin, capsorubin, and capsanthin 5,6 epoxides (9). Capsaicinoids such as capsaicin and dihydrocapsaicin contribute to the characteristic pungency of red pepper. Besides its culinary uses, red pepper is appreciated for its analgesic, anti-obesity, and antimicrobial properties. Capsaicin and other phenolic compounds in red pepper contribute significantly to its antioxidant capacity. Several research have exhibited that red pepper extract is capable of preventing various pathogenic microorganisms from growing, including foodborne pathogens. It has a variety of therapeutic benefits, such

as elevating lipid metabolism, improving digestion, and having a hypocholesterolemia impact on cholesterol and gallstones (10).

Spice saffron (*Crocus sativus*) is made from the dried stigma of the flower, which only makes up 7.4% of the *C. sativus* flowers. Saffron plants contain three dry stigmas that each weigh about 2 mg, so roughly 150,000 flowers need to be meticulously picked to produce 1 kg of spice. Harvesting the flowers and sorting out the stigmas is a laborious process (11, 12). Saffron is primarily produced in Iran and is considered the costliest spice globally. This plant is comprised of a variety of unique compounds, including crocin, crocetin, safranal, and picrocrocin, contributing to its intense color and aroma, and therapeutic properties. Several advantages of saffron have been documented for human health, including preventing cancer and tumors, protecting against dementia, enhancing memory, treating menstrual disorders, and alleviating depression (13, 14). Saffron has antioxidant capabilities due to its inhibition of lipid peroxidation and scavenging of free radicals. There is also evidence of its antimicrobial potential (15, 16).

Nowadays, natural additives have garnered significant interest as alternatives to synthetic preservatives. This research can offer valuable insights into natural plant extracts to encourage the use of bioactive compounds in the food and pharmaceutical industries. In recent years, numerous studies have investigated the antioxidant and antimicrobial properties of plant extracts; however, most of these works have been conducted outside Iraq, and only limited data are available under the country's specific environmental and agricultural conditions. The current study aimed to

compare the antioxidant and antimicrobial activities of ginger, saffron, and red pepper extracts. The antioxidant activity was determined using DPPH radical scavenging. Additionally, the total phenolic contents and total flavonoid contents of extracts were determined. The antimicrobial activities of plant extracts were examined the minimum inhibitory concentration and minimum bactericidal concentration against selected Gram-positive and Gram-negative bacteria.

2. Materials and Methods

2.1. Materials

Plants, including ginger, saffron, and red pepper, were collected from the market in Sulaymaniyah, Kurdistan region of Iraq. DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, ethanol, ascorbic acid, Folin-Ciocalteu, Na_2CO_3 , AlCl_3 solution, quercetin, and dimethyl sulfoxide were purchased from Merck (Darmstadt, Germany). The bacterial strain, including *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 11778), *Salmonella typhimurium* (ATCC 14028), and *Escherichia coli* (ATCC 43888), was obtained from ATCC (Manassas, VA, USA).

2.2. Preparation of ginger, saffron, and red pepper extract

First, the plants were ground thoroughly. Then, 50 g of plant powder was added to 200 mL of distilled water, stirred at 60 °C for 24 h, and filtered via Whatman No.3 (12, 17).

2.3. Antioxidant scavenging of plant extracts

First, different concentrations of extracts were obtained in methanol (0.004, 0.02, 0.1, 0.5, and 1 mg/mL). Then, 1 mL of each was moved to 1 mL of DPPH (0.15 mM

methanolic solution) and left for 30 min in dark. Simultaneously, a control (1 mL DPPH in methanol) was prepared. Additionally, ascorbic acid was prepared as a positive control, similar to the plant extract procedure. Lastly, the absorbance at 517 nm was determined with a UV-Vis spectrophotometer (Ultrospec 2000, Scinteck, UK). The DPPH quenching activity of plant extracts was computed via the following equation (5, 18):

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Ab}_{\text{Control}} - \text{Ab}_{\text{sample}}}{\text{Ab}_{\text{Control}}} \times 100$$

2.4. Total phenolic contents (TPC) of plant extracts

Initially, 50 μL of different extracts (1 dissolved in distilled water) were added to 2.5 mL of 0.1 N Folin-Ciocalteu and 2 mL of 7.5% Na_2CO_3 , then incubated in dark place at 40°C for 30 min. The absorbance was read at 765 nm with a UV-Vis spectrophotometer, and the results expressed as equivalent mg of gallic acid per g of extract (mg GAE/g) (17, 19).

2.5. Total flavonoid contents (TFC) of plant extracts

First, 50 μL of each extract was poured into 1.15 mL ethanol and 50 μL of an AlCl_3 solution, and then kept at 40°C for 30 min in darkness. Quercetin was utilized as a standard. The absorbance at 415 nm was determined with a UV-Vis spectrophotometer. Total flavonoid content was expressed as mg of quercetin equivalent (QE) per g of extract (mg QE/g) (20).

2.6. Antibacterial

To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different plant extracts, the broth microdilution method was employed using a sterile 96-well microplate. The bacterial strains were first cultured in

nutrient broth and incubated at 37°C for 13 h prior to the test. The stock solutions of the extracts were prepared at a concentration of 150 mg/mL in dimethyl sulfoxide. In the first step, 100 µL of nutrient broth medium was poured into the microplate well. Then, 100 µL of each extract was poured into the first and second wells, and then 100 µL was transferred from the second well to the third well, and from the third well to the fourth to ninth wells, and 100 µL of extract was discarded from the ninth well. In the control well, no extract was added. In the next step, 10⁶ cfu/mL of bacteria strain was prepared, and 100 µL of it was poured into wells except the extract control. Then, wells are incubated for turbidity and bacterial growth. The MIC was defined as the lowest concentration of the extract that entirely inhibited visible bacterial growth (i.e., no turbidity). To determine the MBC, 5 µL from the wells that showed no visible growth were subcultured onto Nutrient agar plates and incubated for 24 h at 37°C. The lowest concentration where no bacterial colonies were observed was considered the MBC (21).

2.7. Statistical analysis

The data were analyzed using IBM SPSS v27 with a one-way ANOVA test. Significance was set at $p < 0.05$.

3. Results

The DPPH scavenging activity of ginger, red pepper, and saffron extracts is illustrated in Fig 1. The findings exhibited that at 1 mg/mL concentration of extracts, the increasing order of antioxidant activity was as follows: saffron (78.5%) < ginger (79.3%) < red pepper (83.4%). So, at 1 mg/mL, red pepper with 83.4% DPPH scavenging activity was closest to the ascorbic acid (positive control) DPPH scavenging activity (99.7%). As

shown in Fig. 1, ginger at 0.2, 0.4, 0.6, and 0.8 mg/mL indicates the highest inhibitory effect.

The total phenolic compounds (TPC) and flavonoid compounds (TFC) of plant extracts are indicated in Table 1. The TPC for ginger, red pepper, and saffron was 9.78 ± 1.42, 4.72 ± 0.85, and 6.41 ± 0.63 mg GAE/g, respectively. The TFC for ginger, red pepper, and saffron was 3.74 ± 0.65, 8.78 ± 0.42, and 2.61 ± 0.34 mg QE/g, respectively.

The results of the antibacterial activity of different extracts are provided in Table 2. The saffron extract exhibited MIC values of 6.25, 3.12, 12.5, and 12.5 mg/mL against *S. aureus*, *B. cereus*, *S. typhimurium*, and *E. coli*, respectively. Corresponding MBC values were 12.5, 6.25, 25, and 25 mg/mL, respectively. In comparison, red pepper extract showed MIC values of 12.5, 12.5, 25, and 50 mg/mL, and MBC values of 25, 25, 50, and 100 mg/mL, respectively, against *S. aureus*, *B. cereus*, *S. typhimurium*, and *E. coli*, respectively. Ginger extract demonstrated relatively stronger antimicrobial activity against *S. aureus*, with the lowest MIC and MBC values of 3.12 and 6.25 mg/mL, respectively. For *B. cereus*, *S. typhimurium*, and *E. coli*, ginger exhibited MIC values of 6.25, 12.5, and 25 mg/mL, and MBC values of 12.5, 25, and 50 mg/mL, respectively. These results indicate that all three plant extracts possessed varying degrees of antibacterial activity, with *B. cereus* being the most susceptible and *E. coli* the most resistant. Among the tested extracts, saffron and ginger generally exhibited stronger antimicrobial effects compared to red pepper.

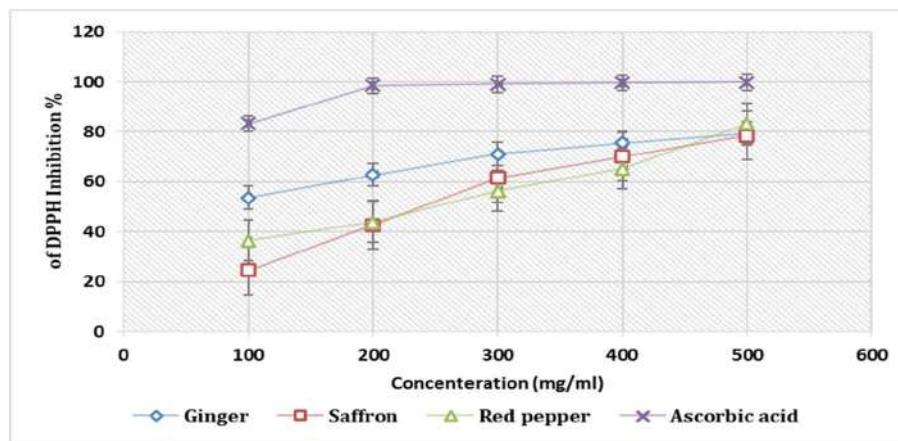


Figure 1. DPPH scavenging activity in the plant extracts (ginger, saffron, and red pepper) and control (ascorbic acid).

Table 1. Total phenolic contents (TPC) and total flavonoid contents (TFC) in plant extracts

Extracts sample	TPC (mg GAE/g)	TFC (mg QE/g)
Ginger	9.78 ± 1.42 ^a	3.74 ± 0.65 ^b
Red pepper	4.72 ± 0.85 ^c	8.78 ± 0.42 ^a
Saffron	6.41 ± 0.63 ^b	2.61 ± 0.34 ^b

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of saffron, red pepper, and ginger extracts against Bacterial pathogens

Bacterial strains	Saffron		Red pepper		Ginger	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>S. aureus</i>	6.25	12.5	12.5	25	3.12	6.25
<i>B. cereus</i>	3.12	6.25	12.5	25	6.25	12.5
<i>S. typhimurium</i>	12.5	25	25	50	12.5	25
<i>E. coli</i>	12.5	25	50	100	25	50

4. Discussion

Plant extracts can donate hydrogen atoms or electrons and capture free radicals due to their antioxidant components. DPPH (stable free radical) was used to assess the antioxidant capacity of the extracts (22). Haddarah et al. (18) reported that at 1 mg/mL ginger had the highest antioxidant activity (93.7%), followed by fennel (89.1%) and borage (86.1%). Karimi et al. (23) found that DPPH scavenging activities of saffron stigma were influenced by the solvent used. They reported that at 300 µg/mL the increasing inhibitory effect of saffron extracts and standards was as follows: ethanol (50.1%) < water (57.6%) < methanol (68.2%) < BHT (89.0%) < α -tocopherol (95.6%). Assimopoulou et al. (24) state that the antioxidant activity of saffron may be linked to crocin and safranal compounds. In another study, DPPH scavenging activities of the water and ethanol extract of ginger and BHA (standard) at 30 µg/mL were 16.2, 43.8, and 74.3%, respectively (7). Maizura et al. (22) found that the DPPH scavenging activity of ginger was 79%. In the study by Mohammad Salamatullah et al. (9), the DPPH scavenging activity of the water extract of red pepper was 70.26%. In another study, the DPPH scavenging activity of red pepper pericarp and red pepper seed at 100 µg/mL was 32% and 13%, respectively (10). The antioxidant feature of the plant extract can be explained by the presence of other bioactive compounds (22).

Phenolic and flavonoid compounds are commonly present as secondary metabolites in plants and have attracted substantial interest due to their various functions. Phenolic compounds have several physiological functions, such as antimicrobial, antioxidant, antimutagenic, and anti-inflammatory. Flavonoids are crucial in the human diet and have

considerable antioxidant activity (14, 23). TPC of ginger in present study is lower its value found by Haddarah et al. (18) (97.36 mg GAE/100 g). Anilakumar et al. (25) reported that TPC of ginger was 13.73 mg GAE/100 g. In another study, TFC for ginger leaves extract (methanol solvent extraction) was 7.05 mg QE/g (26). Karimi et al. (23) found that TPC and TFC of saffron (water extract) was 5.7 mg GAE/g and 3.8 mg rutin equivalent/g, respectively, which is close to our findings. Azghandi Fardaghi et al. (27) found that TFC for saffron stigma was 183.25 g QE/100g. Chen and Kang (28) reported that TPC for different parts of red pepper (methanol extract) ranged from 52.27 to 71.34 mg GAE/g, respectively. They also found that TFC for red pepper pericarp, placenta, and stalk were 46.71, 58.96, and 69.84 narigin equivalents (NAE) mg, respectively (28). In another study, TPC and TFC for red pepper was 28.73 mg GAE/g and \approx 11 mg CE/g, respectively (9). Another study reported that TPC for different pepper varieties (water extract) varied from 55.73 to 138.00 µmol GAE/g, which significantly higher than present results (29). The differences in TPC and TFC might be linked to several factors, such as the extraction solvent, agronomical and environmental factors, varietal differences, the type of soil, and the presence of stress factors (9, 14).

MIC and MBC investigated the antibacterial activity of ginger, saffron, and red pepper extracts concerning the four species of Gram-positive and Gram-negative bacteria. Another study reported that ginger extract had an MIC value of 0.46 mg/mL against *S. aureus* and *B. cereus* and an MIC value of 0.93 mg/mL against *S. typhimurium* and *E. coli*. They also reported that ginger extract against *S. aureus* and *B. cereus* had MBC value of

0.93 mg/mL and against *S. typhimurium* and *E. coli* had MBC value of 1.86 mg/mL (8). Farmoudeh et al. (3) reported that the aqueous extract of ginger had MIC values of 62.5 mg/mL and MBC value of 250 mg/mL against *S. aureus* and *E. coli*. Asgarpanah et al. (11) reported that the saffron extract had MIC values of 62.5, 62.5, 62.5, and 125 mg/mL against *S. aureus*, *B. cereus*, *S. typhi*, and *E. coli*, respectively. While, the MBC value against these bacteria was 250, 125, 125, and 250 mg/mL, respectively. In another study, saffron extract against *S. aureus* (DSM 20231) showed MIC and MBC value of 4.5 and 9 mg/mL, respectively, as well as against *E. coli* (DSM 30083) had MIC and MBC value of 9 and 18 mg/mL, respectively (30). In the study by Mohammad Salamatullah et al. (9), the MIC value of the aqueous extract of red pepper against *S. aureus* and *E. coli* was 2 and 1 mg/mL, respectively. Whereas, the MBC value for these pathogens was 8 and 2 mg/mL, respectively.

5. Conclusion

In recent years, plant extracts have received considerable scrutiny concerning addressing increased antibacterial resistance and as an alternative to synthetic antioxidants. This research was conducted to examine the potential antioxidant and antibacterial capacity of saffron, red pepper, and ginger extracts. The highest antioxidant capacity at 1 mg/mL was found in red pepper extract. Ginger had the highest TPC, and red pepper had the highest TFC. Ginger and saffron extracts showed more potent antimicrobial activity than red pepper. In general, the extracts were most effective against *B. cereus*, and the least effective against *E. coli*. The difference in antioxidant and antibacterial activities in plants is due to the composition of the

extracts, which vary based on location, season, age of the plant, and the growth stage. Our findings exhibit the potential of saffron, red pepper, and ginger extracts as natural sources of antioxidant and antimicrobial compounds. One of the most significant limitations of the present study is the lack of sufficient funding and facilities. Future research is recommended to perform additional tests to identify and characterize the specific components responsible for the antibacterial and antioxidant properties.

Authorship contribution statement

Shilan Muhammad Abdulla: Supervision, conceptualization, Project administration, Formal analysis, Data curation, Investigation, Writing - original draft, Writing - review & editing

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Declaration of competing interest

Authors have no known financial and personal relationships with other people or organizations that could inappropriately influence or bias their work.

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Data availability

The datasets used and/or analyzed during the study are available from the corresponding author upon reasonable request.

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