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Antibacterial Activities of Saffron Petal (*Crocus Sativus L.)* Extract against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*

*Ferdous J[1], Razia S[2], Nishat EF[3], Hossain MA[4], Dema TS[5], Tumpa MP[6]

Abstract

The emergence of antimicrobial resistance necessitates the search for novel antibacterial agents. Saffron (*Crocus sativus L.*) petals, a major by-product of saffron production, are a potential source of bioactive compounds. This experimental study in the Department of Pharmacology and Therapeutics in collaboration with the Department of Microbiology, Mymensingh Medical College, Bangladesh, between July 2022 and June 2023, to evaluate in vitro antibacterial activity of saffron petal extracts against common pathogenic bacteria. Aqueous (ASE) and methanolic (MSE) extracts of saffron petals were prepared. Their antibacterial activity against standard strains of *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli),* and *Pseudomonas aeruginosa (P. aeruginosa)* was assessed using the disc diffusion method to determine the zone of inhibition (ZOI) and the broth dilution method to determine the minimum inhibitory concentration (MIC). Both extracts exhibited concentration-dependent antibacterial activity. The methanolic extract (MSE) demonstrated superior potency. The largest ZOI (24 mm) was observed against *S. aureus* at 500 mg/ml for both extracts. The MIC values for MSE were 50 mg/ml for *S. aureus* and 250 mg/ml for both *E. coli* and *P. aeruginosa*. The MIC values for ASE were 100 mg/ml for *S. aureus* and *E. coli*, and 250 mg/ml for *P. aeruginosa*. *S. aureus* was the most susceptible organism. The positive control, Gentamicin, showed significantly lower MICs (0.75-1 µg/ml), as expected. subculture studies confirmed the bactericidal action at the determined MICs. Saffron petal extracts, particularly the methanolic fraction, possess significant in vitro antibacterial activity. These findings validate the traditional use of this agricultural waste and position saffron petals as a promising, sustainable source for further investigation into novel antimicrobial agents.

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Introduction

The escalating crisis of antimicrobial resistance (AMR) represents one of the most pressing challenges to global public health, rendering many conventional antibiotics increasingly ineffective.[1] This has spurred an urgent need for the discovery of novel antimicrobial agents from alternative sources. In this context, medicinal plants have re-emerged as a promising reservoir of bioactive compounds, offering a rich tapestry of phytochemicals with diverse therapeutic potentials.[2] Saffron (*Crocus sativus Linn.*), derived from the stigmas of the flower, is renowned as much for its culinary value as for its historical use

1. *Dr. Jannatul Ferdous, Assistant Professor, Department of Pharmacology and Therapeutics, Community Based Medical College Bangladesh.
2. Dr. Sultana Razia, Assistant Professor (C.C), Department of Pharmacology and Therapeutics, Community Based Medical College Bangladesh.
3. Dr. Ekra Ferdousi Nishat, Lecturer, (Pharmacology), Netrokona Medical College, Bangladesh.
4. Dr. Mahabuba Azmary Hossain, Assistant Professor (In situ), 250 Bedded General Hospital, Jamalpur.
5. Dr. Taslima Sultana Dema, Lecturer, (Pharmacology), Community Based Medical College Bangladesh.
6. Dr. Monisha Paul Tumpa, Lecturer, (Pharmacology), Community Based Medical College Bangladesh.

Address of Correspondence:
Email: methila2015@gmail.com

in traditional medicine. It has been extensively studied for its pharmacological properties, including antioxidant, antidepressant, and anti-inflammatory activities.[3] However, the cultivation of saffron is a labor-intensive process, generating a significant biomass of by-products, primarily the petals, which are often considered agricultural waste. The sustainable utilization of these by-products presents an opportunity for value addition. Recent investigations have revealed that saffron petals are not mere waste but are a rich source of valuable bioactive compounds, including anthocyanins, flavonoids, and kaempferol derivatives, which contribute to significant antioxidant and antimicrobial activities.[4,5] Several studies have provided preliminary evidence supporting the antibacterial potential of saffron petal extracts. Research has indicated that both aqueous and alcoholic extracts of saffron petals exhibit inhibitory effects against a range of food-borne bacterial pathogens.[6,7] The antimicrobial efficacy is often attributed to the complex mixture of flavonoids and other phenolic compounds present, which can disrupt microbial cell membranes and inhibit essential enzymes.[8] Furthermore, the synthesis of nanoparticles using saffron petal extracts has also demonstrated enhanced antimicrobial properties, highlighting the potential of this material in nanobiotechnology.[9] Among the most concerning pathogens are Staphylococcus aureus, a common cause of skin and soft tissue infections that can lead to bacteremia; Escherichia coli, a frequent agent of urinary tract and gastrointestinal infections; and Pseudomonas aeruginosa, a notoriously multidrug-resistant organism associated with hospital-acquired infections.[10,11] The fight against these pathogens is hampered by their ability to develop resistance mechanisms; searching for new therapeutic options is critical.[12,13] A comprehensive comparative analysis of different extract types against standard reference strains of these critical pathogens, coupled with the determination of minimum inhibitory concentrations (MICs), is essential to validate their therapeutic potential. Therefore, this study aimed to systematically evaluate the in vitro antibacterial activities of aqueous and methanolic extracts of saffron petals (*Crocus sativus L.*) against reference strains of *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*, and to determine their respective MICs to quantify antimicrobial potency.

Methods

This experimental study was conducted in the Department of Pharmacology and Therapeutics in collaboration with the Department of Microbiology, Mymensingh Medical College, Bangladesh, between July 2022 and June 2023.

Plant material and extract preparation: Mature saffron petals (*Crocus sativus L.*) were procured from a local market in Dhaka, Bangladesh. The petals were washed, shade-dried at room temperature for five days, and pulverized into a fine powder using an electric blender. Both aqueous (ASE) and methanolic (MSE) extracts were prepared. For each, 10 grams of powder were soaked in 100 ml of solvent (distilled water or 95% methanol) for 24 hours with continuous stirring, protected from light. The solutions were filtered through Whatman No. 1 filter paper. The filtrates were evaporated to dryness in Petri dishes at 55°C. The resulting dried extracts were reconstituted in 5% dimethyl sulfoxide (DMSO) to achieve a stock solution of 1 g/ml, which was stored at 4°C.

Test microorganisms and standardization: Standard reference strains of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were used.

Bacterial suspensions were prepared in sterile normal saline and adjusted to a turbidity equivalent to the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL).

Antibacterial susceptibility testing: The antibacterial activity of the extracts was evaluated using the Kirby-Bauer disc diffusion method. Briefly, Mueller-Hinton agar plates were inoculated with the standardized bacterial suspensions. Sterile 6 mm filter paper discs were impregnated with 10 µL of each extract concentration (500, 200, 100, 50, 20, 10, and 5 mg/ml) and placed on the inoculated agar. Gentamicin (10 µg/disc) and 5% DMSO served as positive and negative controls, respectively. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured in millimeters.

Determination of minimum inhibitory concentration (MIC): The MIC of the extracts and Gentamicin was determined using the broth dilution technique. Two-fold serial dilutions of the extracts (500-5 mg/ml) and Gentamicin (2-0.25 µg/ml) were prepared in nutrient broth. Each tube was inoculated with 20 µL of the standardized bacterial suspension and incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration that prevented visible bacterial growth. Subculturing from clear tubes onto nutrient agar was performed to confirm bactericidal activity.

Categorical variables were summarized using frequencies and percentages. Associations were examined using unpaired Student's t-test test and two-way ANOVA as appropriate. Statistical analysis was conducted using IBM SPSS version 26.0 for Windows, with level of significance set at <0.05. Data was presented in tabulated form.

As no human or animal was used in the experimentation, this study protocol was exempted from ethical review by the permission of the Institutional Review Board of Mymensingh Medical College, Bangladesh.

Results

The experimental results demonstrated that both aqueous and methanolic extracts of saffron petals possess significant, concentration-dependent antibacterial activity against the tested microorganisms. The methanolic extract consistently showed superior potency compared to the aqueous extract. In the initial disc diffusion assay, the highest concentration of 500 mg/ml of both extracts produced substantial zones of inhibition against all three bacteria. Against *Staphylococcus aureus*, the aqueous and methanolic extracts produced a zone of inhibition of 24 mm each. For *Escherichia coli* and *Pseudomonas aeruginosa*, both extracts produced an 18 mm zone at this concentration. The antibacterial activity diminished with decreasing extract concentrations, with the methanolic extract maintaining significant activity at lower concentrations than the aqueous extract. For instance, against *Staphylococcus aureus*, the methanolic extract produced a zone of inhibition of 13 mm at 50 mg/ml, whereas the aqueous extract required 100 mg/ml to produce a similar effect (Table-I & II). A follow-up experiment determined the minimum concentration required for a definite antibacterial effect, defined as a zone of inhibition larger than 6 mm. For the aqueous extract, the threshold concentrations were 100 mg/ml for *Staphylococcus aureus*, 150 mg/ml for *Escherichia coli*, and 350 mg/ml for *Pseudomonas aeruginosa*. For the more potent methanolic extract, the thresholds were 50 mg/ml for *Staphylococcus aureus* and 350 mg/ml for both *Escherichia coli* and

Pseudomonas aeruginosa (Table-III). Statistical analysis confirmed that the zones of inhibition for both extracts at their active concentrations were significantly larger than the negative control. The broth dilution method provided a quantitative measure of antibacterial potency through the MIC. The results confirmed the greater efficacy of the methanolic extract, particularly against *Staphylococcus aureus*, with a MIC of 50 mg/ml, which was twice as potent as the aqueous extract, which had a MIC of 100 mg/ml. For the Gram-negative bacteria, the minimum inhibitory concentration of the methanolic extract for *Escherichia coli* and *Pseudomonas aeruginosa* was 250 mg/ml each. In comparison, the aqueous extract showed a MIC of 100 mg/ml for *Escherichia coli* and 250 mg/ml for *Pseudomonas aeruginosa*. All MIC results were validated by subculture studies on nutrient agar, which confirmed the bactericidal nature of the inhibition by showing no viable bacterial growth. In contrast, the standard antibiotic gentamicin was vastly more potent, as expected, with MIC values of 0.75 µg/ml for *Staphylococcus aureus* and 1 µg/ml for both *Escherichia coli* and *Pseudomonas aeruginosa* (Table-IV, V & VI).

Table-I: Zone of Inhibition (ZOI in mm) of aqueous saffron extract (ASE) at different concentrations

Concentration (mg/ml)	*S. aureus*	*E. coli*	*P. aeruginosa*
500	24	18	18
200	15	15	9
100	13	8	6
50	6	6	6
20	6	6	6
10	6	6	6
5	6	6	6
Control (5% DMSO)	6	6	6

Two-way ANOVA was done; the effect of concentration on ZOI was statistically significant ($p<0.001$).

Table-II: Zone of Inhibition (ZOI in mm) of methanolic saffron extract (MSE) at different concentrations

Concentration (mg/ml)	*S. aureus*	*E. coli*	*P. aeruginosa*
500	24	18	18
200	22	11	12
100	17	8	8
50	13	6	6
20	6	6	6
10	6	6	6
5	6	6	6
Control (Methanol)	6	6	6

Two-way ANOVA was done; the effect of concentration on ZOI was statistically significant ($p<0.001$).

Table-III: Threshold concentrations for definite antibacterial activity from repeated disc diffusion assays

Test organism	ASE (mg/ml)	ZOI (mm)	MSE (mg/ml)	ZOI (mm)
S. aureus	100	13	50	13
E. coli	150	13	350	13
P. aeruginosa	350	16	350	13

Table-IV: Comparison of antibacterial efficacy (MIC) between extracts and gentamicin

Test Organism	MIC			Potency ratio (MSE vs. ASE)*
	ASE (mg/ml)	MSE (mg/ml)	Gentamicin (µg/ml)	
S. aureus	100	50	0.75	2
E. coli	100	250	1	0.4
P. aeruginosa	250	250	1	1

*Potency Ratio = MIC(ASE) / MIC(MSE). A value >1 indicates MSE is more potent

Table-V: Statistical significance of the zone of inhibition compared to the control

Extract	Concentration (mg/ml)	*S. aureus* p-value	*E. coli* p-value	*P. aeruginosa* p-value
ASE	500	<0.001	<0.001	<0.001
ASE	200	<0.001	<0.001	<0.01
ASE	100	<0.001	<0.05	1.000
MSE	500	<0.001	<0.001	<0.001
MSE	200	<0.001	<0.001	<0.001
MSE	100	<0.001	<0.05	<0.05
MSE	50	<0.001	1.000	1.000

Unpaired Student's t-test was applied to compare ZOI at each concentration to the control (n=3)

Table-VI: Confirmation of MIC results by subculture on nutrient agar

Test organism	ASE MIC (mg/ml)	Subculture result	MSE MIC (mg/ml)	Subculture result	Gentamicin MIC (µg/ml)	Subculture result
S. aureus	100	No growth	50	No growth	0.75	No growth
E. coli	100	No growth	250	No growth	1	No growth
P. aeruginosa	250	No growth	250	No growth	1	No growth

Discussion

The present study provides compelling evidence that saffron petal (*Crocus sativus L.*) extracts possess significant in vitro antibacterial activity, thereby validating the traditional use of this agricultural by-product and highlighting its potential as a source of antimicrobial agents. The findings align with and substantially extend previous research on the bioactivity of this plant material.[15,16] Our results consistently demonstrated that the methanolic extract (MSE) exhibited greater potency than the aqueous extract (ASE), a phenomenon commonly observed in phytochemical research due to methanol's superior efficiency in extracting a broader spectrum of antimicrobial compounds, including flavonoids, alkaloids, and terpenoids.[17] The differential susceptibility observed among the bacterial strains offers critical insights. *Staphylococcus aureus*, a Gram-positive bacterium, was the most susceptible to both extracts, showing the largest zones of inhibition and the lowest minimum inhibitory concentration (MIC) values. This heightened susceptibility can be attributed to the structural difference in the cell envelope; the single peptidoglycan layer in Gram-positive bacteria is more accessible to antimicrobial compounds than the complex outer membrane possessed by Gram-negative bacteria like *Escherichia coli* and *Pseudomonas aeruginosa.*[9,14] The latter two were more resistant, with *Pseudomonas aeruginosa* requiring the highest extract concentrations for inhibition. This intrinsic resistance is well-documented and is largely due to its efflux pump systems and low-permeability outer membrane, which acts as a formidable barrier to foreign molecules.[18] Our quantitative MIC findings are particularly telling. The MIC of the methanolic extract

against Staphylococcus aureus was 50 mg/ml, which is comparable to the efficacy reported in earlier studies on saffron petal extracts against food-borne pathogens.[7,19] The higher MIC values required for the Gram-negative bacteria (250 mg/ml for both extracts against *Pseudomonas aeruginosa*) underscore the challenge in combating such resilient pathogens. While these MIC values are substantially higher than those of the conventional antibiotic gentamicin (MIC 0.75-1 µg/ml), this comparison is not intended to equate the two, but rather to contextualize the activity of the plant extracts. The potency of a purified, single-molecule antibiotic is expected to be far greater than that of a complex crude extract. The significance of our findings lies in the proven antibacterial effect of the extracts themselves, which contain a cocktail of bioactive compounds that can act synergistically to inhibit bacterial growth and potentially mitigate the development of resistance.[20] The confirmation of bactericidal activity through subculture studies from the MIC tests reinforces the reliability of our results, indicating that the extracts at their MIC levels are not merely bacteriostatic but effectively kill the bacteria. This adds a crucial dimension to their potential application. The antibacterial properties are likely mediated by the rich phytochemical composition of saffron petals, which have been previously reported to contain flavonoids like kaempferol, anthocyanins, and other phenolic compounds known for their ability to disrupt microbial cell membranes and inhibit essential enzymes.[8,20,21] This study successfully transforms saffron petals from a waste product into a subject of pharmacological interest. The valorization of such agricultural by-products is not only economically advantageous but also aligns with the principles of sustainable and green chemistry.[17,18] However, the journey from in vitro efficacy to clinical application is a long one. Future research must focus on the bioassay-guided fractionation of these extracts to identify the specific active constituents, assess their safety and toxicity profiles in mammalian cell lines, and evaluate potential synergistic effects with existing antibiotics to combat multidrug-resistant strains.[16]

This study is limited by its in vitro design, which does not reflect in vivo physiological conditions. The high MIC values of the extracts compared to standard antibiotics may also limit their immediate therapeutic applicability. Further pharmacological and toxicological studies are required.

Conclusion

This study conclusively demonstrates that saffron petal extracts, particularly the methanolic fraction, possess significant in vitro antibacterial activity against *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa*. The methanolic extract showed superior efficacy, with Staphylococcus aureus being the most susceptible organism. These findings validate the traditional use of this agricultural by-product and position saffron petals as a promising, sustainable source for further investigation into novel antimicrobial agents. Future research should isolate the specific active compounds within the extracts. Their synergistic effects with conventional antibiotics should be evaluated. Comprehensive in vivo toxicological and pharmacological studies are also essential to assess their potential for clinical development.

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