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Antibacterial Activity of Crude Extract and Solvent Fractions of *Crocus sativus* L. Against Selected Gram-Positive and Gram-Negative Bacteria

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Abstract

The rapid development of the multidrug-resistant (MDR) bacterial pathogens has exacerbated the worldwide quest to obtain alternative antimicrobial agents derived naturally. Saffron (*Crocus sativus L.* (Iridaceae) is a medicinal plant that contains bioactive phytochemicals such crocin, crocetin, safranal and flavonoids. The current research was conducted to determine the antibacterial activity of crude extract and subsequent solvent fractions (n-hexane, chloroform, ethyl acetate, and n-butanol) of *C. sativus* against selected Gram-positive and Gram-negative bacterial isolates using the agar well diffusion technique. The n-butanol fraction of the tested fractions displayed the best antibacterial effect and was active against Bacillus stearothermophilus (29.15 ± 1.43 mm) and Escherichia coli (26.00 ± 1.98 mm), which is close to streptomycin (30.44 ± 0.40 mm). The chloroform fraction was found to be also active against E. coli (25.23 ± 0.87 mm). Conversely, the n-hexane fraction exhibited relatively weak anti-bacterial activities. The findings show that the apparently antibacterial activity is probably due to moderately polar phytoconstituents. The results suggest that C. sativus can be further used as a natural source of antibacterial agents and need to be investigated as a source of bioactive compounds and mechanisms of action.

KEYWORDS

Crocus sativus; antibacterial activity; solvent fractionation; multidrug resistance; phytochemicals; Gram-positive bacteria; Gram-negative bacteria

1.0 INTRODUCTION

The development and quick spread of antimicrobial resistance (AMR) is one of the most severe threats to the populace all over the world [1]. Clinical and agricultural use of antibiotics has increased the selection of resistant strains, such as extended-spectrum β-lactamase (ESBL)-producing Escherichia coli, methicillin-resistant Staphylococcus aureus (MRSA), and carbapenem-resistant Klebsiella pneumoniae [2]. The progressive action of traditional antibiotics requires the urgent identification of new groups of antimicrobial substances, which have different mechanisms of action. The historical role of natural products as an indispensable source of drug discovery has proven to be of great importance. About 70 percent of the existing antimicrobial agents are natural

products or natural scaffolds [3]. Among the most commonly occurring structurally diverse secondary metabolites are alkaloids, terpenoids, phenolics, flavonoids, glycosides, and carotenoids, many of which have strong antimicrobial action and are found in medicinal plants (especially). The compounds can have antibacterial action through various mechanisms that include membrane disruption, nucleic acid synthesis, and disrupting metabolic pathways, and oxidative stress [4].

Saffron is a perennial plant of the family Iridaceae that is also called crocus sativus L. It is commonly grown in Iran, India, Spain and even in the Central Asia. In addition to culinary and economic value, C [5]. sativus has been widely used in the traditional medicine of respiratory diseases, gastroenterology, inflammation, and infectious disease treatment [6]. Phytochemical studies of C. sativus have revealed a number of bioactive compounds with crocin (carotenoid glycoside), crocetin and picrocrocin being the predominant ones [7]. Moreover, the plant also contains flavonoids and phenolic acids which make it have antioxidant and antimicrobial effects [8]. Recent reports have indicated that crocin and safranal possess bacteriostatic and bactericidal action by changing the membrane permeability and by inhibition of mandatory enzymatic systems [9, 10].

Although the pharmacological properties of saffron have been widely explored, comprehensive studies comparing the antibacterial activity of different solvent fractions remain limited. Solvent fractionation based on polarity enables the selective extraction of specific classes of phytochemicals, thereby facilitating the identification of bioactive fractions. Moreover, understanding the differential activity of polar versus non-polar fractions provides insight into the chemical nature of antimicrobial constituents.

Therefore, the present study was designed to evaluate the antibacterial potential of crude extract and successive solvent fractions of *Crocus sativus* against clinically relevant Gram-positive and Gram-negative bacterial strains. The study aims to identify the most active fraction and to provide a scientific basis for future phytochemical isolation and mechanistic investigations.

2.0 MATERIAL AND METHODS

2.1 Plant Collection and Identification

Fresh plant material of Crocus sativus L. was taken in an agricultural farm area. Taxonomic authentication of the plant was done by a qualified botanist, and a voucher was archived in the departmental herbarium to be used in the future. The material collected was washed well using distilled water to wash away soil and debris and dried under shade at room temperature (25-30 ^{o}C) in two weeks to avoid degradation of volatile compounds. With the help of a mechanical grinder, the dried material was finely ground into a fine powder and then stored in light and moisture-free containers.

2.2 Extraction

About 500g of the powdered plant materials were impregnated in 2.5 L of methanol and macerated over 72 hours and at room temperature with occasional shaking to allow maximum phytoconstituent extraction. Whatman No. 1 filter paper was used to filter the mixture. To prepare a semi-solid crude methanolic extract, a rotary evaporator at 40 ^{o}C was used to concentrate the filtrate under reduced pressure. The extract was kept at 4 ^{o}C until further fractionation.

2.3 Fractionations

Solvents Successive solvent-solvent partitioning was done on the crude methanolic extract of *Crocus sativus* to partition phytoconstituents according to their polarities. The dried crude extract was suspended in distilled water and passed to separate funnel to extract with liquid. Fractionation was done consecutively in decreasing order of solvents of lower polarity (n-hexane, chloroform, ethyl acetate, and finally n-butanol). The solvents were carefully measured and mixed and left to stand until both phases were distinct. The corresponding layers of the organic layer were obtained separately and the extraction procedure was repeated a number of times to accomplish optimum recovery of bioactive compounds. The collected fractions were pooled at less pressure via a rotary evaporator at constant temperature to avoid the decomposition of volatile compounds. Dried fractions were then weighed and stored under 4 ^{o}C, in airtight containers and awaited further antibacterial analysis.

2.4 Antibacterial Activities

The antibacterial activity of the crude extract and solvent fractions of *Crocus sativus* was evaluated against selected Gram-positive and Gram-negative bacterial strains,

including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptodirimu* sp., and *Bacillus stearothermophilus*. Pure bacterial cultures were maintained on nutrient agar slants at 4°C and subcultured prior to experimentation to ensure viability and purity. For the preparation of inoculum, individual colonies were transferred into sterile nutrient broth and incubated at 37°C for 18–24 hours. The antibacterial activity was determined using the agar well diffusion method. Sterile Mueller–Hinton agar plates were prepared and uniformly inoculated with the standardized bacterial suspension using sterile cotton swabs to obtain a confluent lawn of growth. Wells of 6 mm diameter were aseptically bored into the agar using a sterile cork borer. 50 µL of each extract and solvent fraction, was carefully introduced into the respective wells. Streptomycin served as the positive control, while the corresponding solvent was used as a negative control to confirm that observed inhibition was attributable solely to the plant extract. The inoculated plates were incubated at 37°C for 24 hours under aerobic conditions. Following incubation, the diameter of the zones of inhibition, including the well diameter, was measured in millimeters using a calibrated digital caliper. All experiments were conducted in triplicate, and the mean inhibition zones were calculated to ensure reproducibility and reliability of the results.

2.5 Statical analysis

All experiments were carried out in triplicate, and results were expressed as mean ± standard deviation (SD).

3.0 RESULTS

3.1 Antibacterial Activity of Crude Extract and Solvent Fractions

The antibacterial activity of crude extract and successive solvent fractions of *Crocus sativus* was evaluated using the agar well diffusion assay, and the results are summarized in **Table 1.** The inhibitory effects varied significantly depending on both the solvent fraction and the bacterial strain tested.

Among all tested fractions, the n-butanol fraction exhibited the highest antibacterial activity against most bacterial strains. The strongest inhibition was observed against *Bacillus stearothermophilus* (29.15 ± 1.43 mm), which was comparable to the positive control streptomycin (30.44 ± 0.40 mm). The butanol fraction also demonstrated strong activity against *Escherichia coli* (26.00 ± 1.98 mm) and *Staphylococcus aureus* (25.43 ± 1.05 mm), indicating broad-spectrum antibacterial potential.

The chloroform fraction showed considerable antibacterial activity, particularly against *E. coli* (25.23 ± 0.87 mm) and *B. stearothermophilus* (27.30 ± 0.43 mm). Moderate inhibition was also observed against *S. aureus* (18.44 ± 0.80 mm). In contrast, the ethyl acetate fraction exhibited moderate activity across all tested strains, with inhibition zones ranging from 16.09 ± 0.55 mm to 24.13 ± 0.22 mm. The n-hexane fraction demonstrated comparatively weak antibacterial activity against all tested bacteria. The lowest inhibition was recorded against *Klebsiella pneumoniae* (7.31 ± 1.22 mm), suggesting limited contribution of non-polar compounds to antibacterial action.

Statistical analysis (one-way ANOVA) revealed significant differences ($p < 0.05$) between solvent fractions for each bacterial strain, indicating that antibacterial activity was strongly influenced by solvent polarity and the nature of extracted phytochemicals.

Table 1: Antibacterial screening of crude extracts and various fractions of *Berberis vulgaris*

Bacterial strain	n-hexane	Chloroform	Ethyl Acetate	Butanol	Streptomycin
E. coli	14.14±1.00	25.23±0.87	24.13±0.22	26.00±1.98	28.76±0.87
S. aureus	12.54±1.20	18.44±0.80	19.67±0.43	25.43±1.05	30.43±0.65
K. pneumonia	7.31±1.22	14.09±0.44	16.09±0.55	20.31±1.44	26.98±0.34
Straptodirimu	11.12±1.23	13.45±0.35	18.44±0.45	19.15±1.23	32.87±0.56
B. stearothermophihus	8.34±1.98	27.30±0.43	22.09±0.60	29.15±1.43	30.44±0.40

3.2 Comparative Susceptibility of Bacterial Strains

A comparative analysis of bacterial susceptibility revealed that Gram-positive bacteria were generally more sensitive

to the plant fractions than Gram-negative bacteria. For instance, *B. stearothermophilus* exhibited high susceptibility to both butanol (29.15 ± 1.43 mm) and chloroform (27.30 ± 0.43 mm) fractions. Similarly, *S. aureus* showed strong inhibition in the presence of the butanol fraction (25.43 ± 1.05 mm). In contrast, Gram-negative strains such as *K. pneumoniae* displayed relatively lower inhibition zones across all fractions, with the highest activity observed in the butanol fraction (20.31 ± 1.44 mm). The reduced susceptibility of Gram-negative bacteria may be attributed to the presence of an outer lipopolysaccharide membrane that acts as a permeability barrier, limiting the penetration of phytochemicals. Interestingly, *E. coli* showed relatively high sensitivity to chloroform (25.23 ± 0.87 mm) and butanol (26.00 ± 1.98 mm) fractions, suggesting that certain phytoconstituents in these fractions possess the ability to penetrate Gram-negative bacterial membranes effectively.

3.3 Influence of Solvent Polarity on Antibacterial Activity

The antibacterial performance of the fractions followed the general order are as follows:

Butanol > Chloroform > Ethyl acetate > n-Hexane

This pattern suggests that moderately polar to polar phytochemicals are primarily responsible for the observed antibacterial activity. The strong activity of the butanol fraction indicates enrichment of polar compounds such as glycosides, flavonoids, and phenolic derivatives, which are well-documented for their antimicrobial properties. The comparatively lower activity observed in the n-hexane fraction suggests that non-polar constituents, including lipids and some terpenoids, may have limited antibacterial efficacy against the tested strains.

3.4 Comparison with Standard Antibiotic

Although streptomycin exhibited superior antibacterial activity overall, the inhibition zones produced by the butanol and chloroform fractions against certain strains approached those of the standard antibiotic. For example, the butanol fraction showed 29.15 ± 1.43 mm inhibition against *B. stearothermophilus*, compared to 30.44 ± 0.40 mm for streptomycin. This result indicates that *Crocus sativus* contains bioactive compounds with substantial antibacterial potency. While plant extracts typically require higher concentration than synthetic antibiotics to achieve comparable effects, the observed inhibition zones highlight the therapeutic promise of saffron-derived phytochemicals.

4.0 DISCUSSION

The present study demonstrates that *Crocus sativus* possesses significant antibacterial activity, with marked variation among solvent fractions. The observed differences in inhibitory potential are strongly associated with solvent polarity, suggesting that antibacterial phytoconstituents are selectively enriched in specific fractions. Among all tested fractions, the n-butanol fraction exhibited the highest antibacterial activity against the majority of bacterial strains. The pronounced inhibition observed against *Bacillus stearothermophilus* (29.15 ± 1.43 mm) and *Escherichia coli* (26.00 ± 1.98 mm) indicates that polar phytochemicals are primarily responsible for the antibacterial effects. Butanol, being a polar organic solvent, is known to extract glycosides, phenolic acids, flavonoids, and certain carotenoid derivatives. These classes of compounds have been extensively reported for their antimicrobial properties, largely attributed to their ability to disrupt microbial membranes and interfere with intracellular metabolic processes.

The chloroform fraction also demonstrated strong antibacterial activity, particularly against *E. coli* and *B. stearothermophilus*. Chloroform typically extracts moderately non-polar compounds such as alkaloids and certain terpenoids. The significant inhibition observed in this fraction suggests that non-polar or semi-polar secondary metabolites also contribute to the overall antibacterial potential of *C. sativus*. In contrast, the n-hexane fraction displayed comparatively weak antibacterial activity across all tested strains. This indicates that highly non-polar constituents, including lipids and waxes, are less likely to play a major role in antibacterial action. The differential susceptibility observed between Gram-positive and Gram-negative bacteria is consistent with established microbiological principles. Gram-negative bacteria possess an outer membrane composed of lipopolysaccharides that acts as a selective permeability barrier, limiting the entry of hydrophobic and high-molecular-weight compounds. Conversely, Gram-positive bacteria lack this outer membrane and have a thicker peptidoglycan layer, which is more accessible to bioactive phytochemicals. In the present study, Gram-positive strains such as *B.*

stearothermophilus and *S. aureus* exhibited higher sensitivity compared to *K. pneumoniae*. However, the notable inhibition of *E. coli* by chloroform and butanol fractions suggests that certain constituents of *C. sativus* can effectively penetrate Gram-negative bacterial membranes.

The antibacterial activity of *Crocus sativus* may be attributed to its well-documented phytochemical profile. Crocin and crocetin, the major carotenoid glycosides in saffron, possess antioxidant and antimicrobial properties. Safranal, a volatile monoterpene aldehyde, has been reported to exhibit bacteriostatic effects through membrane destabilization and interference with cellular respiration. Flavonoids and phenolic acids present in saffron may exert antibacterial effects by forming complexes with extracellular and soluble proteins, disrupting microbial cell walls, inhibiting nucleic acid synthesis, and generating oxidative stress within bacterial cells. The inhibition zones produced by the butanol fraction against *B. stearothermophilus* approached those of the standard antibiotic streptomycin. While synthetic antibiotics generally demonstrate greater potency due to their targeted mechanisms, the comparable activity observed in this study highlights the therapeutic potential of plant-derived compounds. Unlike conventional antibiotics that often act through single molecular targets, phytochemicals typically exert multi-target effects, which may reduce the likelihood of rapid resistance development.

The observed consistency of inhibition zones, reflected by relatively low standard deviation values, further supports the reproducibility and reliability of the experimental findings. The results align with previous reports suggesting that saffron extracts exhibit moderate to strong antibacterial activity depending on extraction method and solvent polarity.

Despite the promising results, it is important to note that agar well diffusion provides preliminary qualitative and semi-quantitative evaluation of antibacterial activity. The diffusion of compounds in agar is influenced by molecular size and solubility, which may affect zone diameters. This results substantiate the antibacterial potential of *Crocus sativus*, particularly its polar fractions, and provide a scientific basis for further exploration of saffron-derived bioactive compounds as alternative antimicrobial agents.

5.0 CONCLUSION

This study demonstrates that *Crocus sativus* possesses notable antibacterial activity against both Gram-positive and Gram-negative bacteria. Among the tested fractions, the n-butanol fraction exhibited the strongest inhibitory effects, indicating that polar phytoconstituents are primarily responsible for the observed antimicrobial activity. The results support the potential of *C. sativus* as a natural source of antibacterial agents. Further investigations, including MIC determination and bioassay-guided isolation of active compounds, are required to validate its therapeutic applicability.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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