

Antibacterial Activity of Aqueous and Methanolic Extracts of *Crocus sativus* Stigma and *Cinnamomum cassia* against Clinical Isolates of some Gram-Positive and Gram-Negative Pathogenic Bacteria

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ABSTRACT

Background and Objectives: Medicinal and aromatic plants are sources of natural antimicrobial compounds that could be useful replacements for antibiotics. The aim of this study was to assess antimicrobial activity of *Crocus sativus* stigma and *Cinnamomum cassia* extracts against some Gram-positive and Gram-negative bacteria.

Methods: Antimicrobial activity of methanolic and aqueous extracts of the plants was tested against clinical isolates of *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus* using the microdilution method. Minimal inhibitory concentration (MIC) and minimum bactericidal concentration of each extract against the mentioned bacteria were also determined.

Results: The MIC of the methanolic extract of *C. cassia* was 80 µg/ml against *Enterococcus*, *K. pneumonia* and *E. coli*. The MIC of the methanolic extract of *C. sativus* was 160 µg/ml against *Enterococcus* and *S. aureus*. The minimum bactericidal concentration of the methanolic extracts of *C. sativus* and *C. cassia* was 320 µg/ml against *K. pneumonia* and 160 µg/ml against *Enterococcus*.

Conclusion: The extracts of *C. sativus* and *C. cassia* exhibit promising antibacterial activities against clinical isolates of the tested bacteria. Our results suggest that the extract of these plants can be further exploited as potential antibacterial agents against multi-drug resistant bacteria.

Keywords: *Cinnamomum aromaticum*, *Crocus*, Anti-Bacterial Agents.

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INTRODUCTION

Infections caused by pathogenic bacteria have been considered a main cause of morbidity and mortality in humans (1), particularly following the emergence and spread of multidrug-resistant bacteria which have increased the risk of treatment failure (2). Hence, it is crucial to find alternative antibacterial agents with therapeutic potential against antibiotic-resistant pathogens. The discovery and use of medicinal plants go back to prehistoric times (3). It is well-demonstrated that extract of some medicinal plants possess antibacterial, antimutagenic, antithrombotic and vasodilatory properties (4). Therefore, medicinal plants are currently investigated as a major source of natural pharmaceutically valuable drugs that could be used for treatment of human diseases (5).

Crocus sativus, commonly known as saffron crocus, is a plant species from the family *Iridaceae* that is native to Southern Europe but currently cultivated worldwide, predominantly in Iran, Spain, Italy and Greece (6). Saffron is the stigma of the plant, which has been used for treatment of several medical conditions, such as gastrointestinal disorders, urinary tract infections, catarrhal and malignancies (7). Cinnamon is another well-known medicinal plant that belongs to the genus *Cinnamomum*, family *Lauraceae*, which is widely distributed in China, India and Australia (8). *Cinnamomum cassia* has been shown to have choleric, anti-ulcer, antifungal, antibacterial, antiviral and anti-inflammatory properties (9). *Cinnamomum* bark is rich in cinnamaldehyde, a highly electronegative compound that inhibits growth of microorganisms by interfering in biological processes involving electron transfer and interacting with nitrogen-containing components, e.g. proteins and nucleic acids (10). The aim of this study was to assess antimicrobial activity of *C. sativus* stigma and *C. cassia* extracts against some Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

C. sativus (stigma) and *C. cassia* were obtained from a local herbal market in Torbat-e Heydariyeh and Mashhad, Iran. The plants were shade dried at room temperature. Dried plants were ground to fine powder using a

homogenizer. Methanolic and aqueous extracts of the plants were obtained by soaking 5 g of plant powder into 50 mL of denatured MeOH (95%) and sterile distilled water in a flask for 72 hours, respectively. The flasks were agitated daily. Stock concentrations of 100 mg/mL of dry extracts in dimethyl sulfoxide were prepared, sterile filtered (0.2 µm) and stored in darkness at 25 °C.

Clinical isolates of Gram-positive (*Staphylococcus aureus*, *Enterococcus* and *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*) bacteria were obtained from patients hospitalized in the Ghaem hospital, Mashhad, Iran. The bacteria were grown overnight in brain heart infusion (BHI) broth (LioFilchem, Italy) at 37 °C. After obtaining a turbidity equivalent to that of a 0.5 McFarland standard (10^8 CFU/mL), bacterial suspensions were adjusted with sterile saline to a concentration of 1×10^5 CFU/ml.

Minimum inhibitory concentration (MIC) of the extracts against the bacteria was determined using the microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) (11). The MIC was defined as the lowest concentration of extract that inhibited growth of bacteria (12). The stock solutions of both extracts were diluted and transferred into first well of a 96-well plate, and serial dilutions were made to obtain concentration range of 80–320 µg/ml. The plate was incubated at 37 °C for 24 hours in aerobic conditions. Then, 200 µl of tetrazolium chloride solution (0.5%) were added to all wells. The plates were examined for color change after 3 hours. To confirm the MIC and minimum bactericidal concentration (MBC), 50 µl were taken from wells with no visible growth and inoculated in Müller-Hinton agar. After 24 hours of aerobic incubation at 37 °C, the numbers of viable microorganisms was determined.

The MBC was then recorded as the lowest concentration that killed at least 99.99% of bacteria (13). All experiments were repeated at least three times. The same concentrations of ethanol and bacteria without the extracts were used as the negative and positive controls, respectively.

RESULTS

Table 1 presents the MIC and MBC values of the methanolic and aqueous extract of *C. sativus* stigma against the tested bacteria. The MIC of the methanolic extract of *C. sativus* stigma was 320 µg/ml against *P. aeruginosa*, *S. pyogenes*, *E. coli* and *K. pneumonia* and 160 µg/ml against *S. aureus*

and *Enterococcus*. The MBC of this extract was 160 µg/ml against *Enterococcus* and 320 µg/ml against other bacteria.

The MIC of the aqueous extract of *C. sativus* was 320 µg/ml or higher against all tested bacteria except for *E. coli* (160 µg/ml).

Table 1- MIC and MBC of the methanolic and aqueous extracts of *C. sativus* stigma

Microorganism	Methanolic extract		Aqueous extract	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
<i>P. aeruginosa</i>	320	320	>320	>320
<i>Enterococcus</i>	160	160	>320	>320
<i>K. pneumonia</i>	320	320	>320	>320
<i>S. pyogenes</i>	160	320	320	320
<i>E. coli</i>	320	320	160	320
<i>S. aureus</i>	160	320	320	640

Table 2 shows the MIC and MBC values of the methanolic and aqueous extracts of *C. cassia* against the tested bacteria. The MIC of the methanolic extract of *C. cassia* was 80 µg/ml against *Enterococcus*, *K. pneumonia*

and *E. coli* and 160 µg/ml against *P. aeruginosa*, *S. pyogenes* and *S. aureus*. According to the results, *P. aeruginosa*, *Enterococcus*, *S. pyogenes*, and *S. aureus* were resistant to the aqueous extract of *C. cassia* (Table 2).

Table 2- MIC and MBC values of the methanolic and aqueous extracts of *C. cassia*

Microorganism	Methanolic extract		Aqueous extract	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
<i>P. aeruginosa</i>	160	320	>320	>320
<i>Enterococcus</i>	80	160	>320	>320
<i>K. pneumonia</i>	80	320	320	640
<i>S. pyogenes</i>	160	320	>320	>320
<i>E. coli</i>	80	160	320	320
<i>S. aureus</i>	160	160	>320	>320

DISCUSSION

Extensive antibiotic use over the years has led to the emergence and spread of antibiotic-resistant bacterial strains. Plant-derived antimicrobial compounds with antibacterial properties could be suitable alternatives to antibiotics for treatment of infections caused by antibiotic-resistant bacteria. In this study, we examined the inhibitory activity of *C. sativus* stigma and *C. cassia* extracts against some Gram-positive and Gram-negative bacteria. The results showed that both plant extracts exhibit varying degree of inhibitory effects on the clinically isolated bacteria. The MIC of the methanolic and aqueous extracts of *C. sativus* stigma against *E. coli* was 320 µg/ml and 160 µg/ml, respectively. In a previous study, the MIC of *C. sativus* stigma extract against *P. aeruginosa* and *E. coli* was 300 µg/ml and 500 µg/ml, respectively. In addition, the MIC and MBC of the extract against *S. aureus* was 200 µg/ml

and 300 µg/ml, respectively (14). However, in our study, the MIC of the methanolic and aqueous extracts of *C. sativus* stigma against *S. aureus* was 160 µg/ml and 320 µg/ml, respectively.

In a study by Pintado et al., the MIC of safranal against *S. aureus* and *E. coli* was 4 mg/ml (15). Methanolic extracts of various *Crocus* spp. were found to have significant inhibitory activity against different bacteria (16). The antimicrobial properties of saffron extracts are mainly attributed to safranal and crocin (17), while cinnamaldehyde is the main active antibacterial constituent of cinnamon (18). In our study, the MIC of the methanolic extract of *C. cassia* was 160 µg/ml against *P. aeruginosa*. Inconsistent with this finding, Gupta et al. claimed that *P. aeruginosa* was resistant to *C. cassia* extract. The MIC values reported by Gupta et al. for *S. aureus* (62.5 mg/ml) and *E. coli* (1000 mg/ml) were also

significantly higher than the values we found in the present study (18). In another study, the ethanolic extracts of cinnamon and turmeric effectively inhibited growth of *Bacillus subtilis* and *E. coli*, which implies that the extracts could be equally effective against both Gram-negative and Gram-positive bacteria (19).

In our study, the methanolic extract of *C. sativus* was more effective than the aqueous extract against growth of *S. pyogenes* and *S. aureus*. Given the results of the MIC assay, it can be concluded that the methanolic extract of *C. cassia* was more effective than the methanolic extract of *C. sativus* against *Enterococcus*, *K. pneumonia* and *E. coli*.

CONCLUSION

The extracts of *C. sativus* and *C. cassia* exhibit promising antibacterial activities against clinical isolates of *S. aureus*, *E. coli*, *P.*

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aeruginosa, *Enterococcus*, *S. pyogenes* and *K. pneumoniae*. Based on the results of the MIC assay, it can be inferred that the methanolic extracts are generally more effective than the aqueous extract against the tested pathogens. Our results suggest that the extract of these plants can be further exploited as potential antibacterial agents against multidrug-resistant bacteria.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest regarding the publication of this article.

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