Bitlis Eren Üniversitesi Fen Bilimleri Dergisi
BİTLİS EREN UNIVERSITY JOURNAL OF SCIENCE
ISSN: 2147-3129/e-ISSN: 2147-3188
VOLUME: 12 NO: 2 PAGE: 496-500 YEAR: 2023
DOI:10.17798/bitlisfen.1248323

Determination of MIC and MBC Values Using Different Extraction Methods in Plants of *Nigella Sativa, Cuminum Cyminum* and *Pimpinella Anisum L.* Samples from Kırıkkale Region

Eftal BÖKE[1*], Birgül KAÇMAZ[2], Aysun ERGENE[3]

[1]*Address (Kırıkkale University, Institute of Science, Department of Biology Kırıkkale, Turkey)*
[2]*Address (Kırıkkale University, department of infectious diseases and clinical microbiology Kırıkkale, Turkey)*
[3]*Address (Kırıkkale University, Faculty of Arts and Sciences, Department of Biology Kırıkkale, Turkey)*
(ORCID: 0000-0002-7052-2790) (ORCID: 0000-0002-5190-7249) (ORCID: 0000-0003-4104-5697)

Keywords: *Nigella Sativa, Cuminum Cyminum, Pimpinella Anisum* , MIC, MBC, methanol

Abstract

The objective of our study was to investigate how 6 plant extracts derived from three plant species exhibit antimicrobial characteristics. The extraction process was conducted using methanol and employed both maceration and soxalet extraction methods. The liquid microdilution method was utilized to assess the ability of Black Cumin (*Nigella sativa*), Cumin (*Cuminum cuminum*), and Anise (*Pimpinella anisum*) cultivated in Kırıkkale province to combat *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). All of the extracts of *Nigellla sativa* (black cumin), *Cuminum cuminum* (Cuminum) and *Pimpinella anisum* (Anise) prepared by the maceration and soxalet extraction method showed antibacterial effects against both bacteria (inhibitory and bactericidal). it was determined that the antibacterial effect of the extracts obtained by the maceration method was higher than the extracts obtained by the soxalet extraction method.

1. Introduction

Herbal medicines have been employed for ages to treat different diseases. The practice of using plant extracts as medicine saw an increase in popularity during the late 1990s. Medicinal plants are important in the search for new drugs, especially in nations that are still developing [1]. There have been numerous efforts to discover new antimicrobial compounds from natural sources.As of recent, the issue of antibiotic-resistant organisms resulting from ineffective chemotherapy has become more prevalent. Stopping the spread of these organisms and enhancing treatment approaches is of utmost importance [2]. The search for more effective drugs and new targets for drug development is becoming increasingly imperative. Although there has been advancement in drug discovery through chemical means such as molecular modeling, computational chemistry, and green synthesis, natural products have demonstrated the value of medicinal plants in terms of their potential to be used for human medicine [3]. A number of recent studies have shown that medicinal plants exhibit various pharmacological and biological properties [4]. Cultivation of medicinal and aromatic plants is a new alternative for producers in Kırıkkale Province. According to literature, three commonly grown plants in Kırıkkale province, namely Black Cumin (*Nigella sativa*), Cuminum (*Cuminum cuminum*), and Anise (*Pimpinella anisum*), have significant medicinal properties and have been traditionally used to treat various ailments throughout history [5-7]. The soil structure and climate of Kırıkkale province are very suitable for the continuous cultivation of these plants. These plants are used in traditional therapy, especially in liver

* Corresponding author: eftal_boke@hotmail.com
Received: 06.02.2023, Accepted: 06.08.2023

diseases, digestive system diseases such as diarrhea, as an appetite enhancer, analgesic and antibacterial. This research aimed to investigate the antimicrobial properties of plant samples in Kırıkkale region of Turkey, such as *Nigella sativa* (black cumin), *Cuminum cuminum* (cumin), and *Pimpinella anisum* (anise), taking into account the geographical conditions of the region.

2. Material and Method

2.1. Plant Examples

Nigellla sativa (black cumin), *Cuminum cuminum* (cumin) and *Pimpinella anisum* (anise) plants grown in Keskin, Karakeçili and Delice districts of Kırıkkale province, respectively, were obtained from Kırıkkale Provincial Directorate of Food, Agriculture and Livestock. It was used in the study aftır species identification was made in Ankara University Faculty of Agriculture. The plants were cleansed using deionized water to get rid of any contaminants or dust and then air-dried in a shaded area at room temperature. The seeds, which are the most used part of the dried plants for consumption, were sieved aftır grinding with a grinding device and the powders with a particle size of 0.50–1.00 mm was used to obtain the plant extract.

2.2. Preparation of Plant Extracts

Plant extracts were obtained by using methanol as a solvent from seeds that were powdered by maceration method. For this purpose, 30 g of plant samples were weighed and 100 mL of methanol solvent was added and kept in a magnetic stirrer at a temperature suitable for the boiling point of the solvent for 24 hours. Plant extracts were obtained aftır filtration. In the soxalet extraction method, 30 g of the plant samples were weighed and 100 mL of solvent was added and they were kept in a soxalet device at a temperature suitable for the boiling point of the solvent. Plant extracts were obtained aftır filtration. Dry plant extracts were dissolved with 10% dimethylsulfoxide (DMSO) and sterilized with membrane filters (0.45 μm) and used for antimicrobial activity studies.

2.3. Evaluation of Antibacterial Activity

This study aimed to investigate the antibacterial activity of methanolic extracts obtained from the seeds of *Nigella sativa* (Black cumin), *Cuminum cuminum* (Cumin), and *Pimpinella anisum* (Anise) using the liquid microdilution approach [8-9]. 100 mg of each extract was weighed and dissolved in 1000 μL of 10% dimethylsulfoxide (DMSO) in an Eppendorf tube. Nutrient broth was used to culture *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) for a period of 24 hours at 37°C, then transferred to nutrient agar and incubated for another 24 hours. The liquid microdilution test was performed by adding 100 μL of Tryptic soy broth to each of the 96 wells on a microplate, followed by 100 μL of the plant extract. The extract concentrations were serially diluted twice to produce a range of values between 5 mg mL^{-1} and 0.0098 mg mL^{-1}.The study involved the addition of 5 μL of bacterial suspensions to each well, which resulted in a final concentration of 5x105 cfu mL^{-1}. To monitor the growth of bacteria, a well without any plant extract was designated as a positive control, and a separate well that only contained plant extract was used as a negative control to evaluate the impact of the plant extract by itself.In this study, the procedure was repeated 4 times for all samples. The microplates were kept in an incubator for a period of 24 hours, the temperature was maintained at 37°C. To identify the minimum inhibitory concentration, the quantity of plant extract that could prevent bacterial growth after the incubation period was determined, and the smallest amount was selected (MIC). A 10 μL sample was taken from the wells with MIC values and concentrations above it, and then inoculated into a blood medium. The inoculated blood medium was incubated at 37°C for another 24 hours. The quantity of bacterial colonies was assessed by counting them upon the completion of the incubation period, and the minimum bactericidal concentration (MBC) was determined, which refers to the concentration that can kill 99.9% of the first living bacteria.

3. Results and Discussion

The efficacy of antibacterial agents derived from *Nigella sativa* (Black cumin), *Cuminum cuminum* (Cumin), and *Pimpinella anisum* (Anise) seed extracts prepared using methanol by the maceration and Soxalet techniques were evaluated for their effectiveness against both gram-positive bacteria, such as *Staphylococcus aureus*, and gram-negative bacteria, including *Escherichia coli*. The broth microdilution technique was used to determine the ability of the antibacterial agents to destroy the bacteria cells.

Tablo 1. MIC and MBC values of *Nigellla sativa* (black cumin), *Cuminum cuminum* (Cuminum) and *Pimpinella anisum* (Anise) seed extracts prepared in methanol by maceration and soxalet extraction method

		Maceration		Soxhlet	
Bacteria	**Extraction Plants**	**MIC (mg/m L^{-1})**	**MBC (mg/m L^{-1})**	**MIC (mg/m L^{-1})**	**MBC (mg/m L^{-1})**
Staphylococcus aureus	***Nigellla sativa***	***0.0625***	***0.0625***	***0.125***	***0.25***
	Cuminum cuminum	***0.125***	***0.5***	***0.25***	***0.5***
	Pimpinella anisum L.	***0.0625***	***0.125***	***0.0625***	***0.125***
Escherichia coli	***Nigellla sativa***	*0.0625*	*0.0625*	*0,125*	*0.25*
	Cuminum cuminum	***0.125***	***0.25***	***0.5***	***0.5***
	Pimpinella anisum L.	***0.125***	***0.25***	***0.125***	***0.5***

MIC and MBC values of *Nigellla sativa* (Black cumin), *Cuminum cuminum* (Cuminum) and *Pimpinella anisum* (Anise) seed extracts prepared in methanol by maceration and Soxalet method are given in Table 1. All of the seed extracts of *Nigellla sativa* (black cumin), *Cuminum cuminum* (Cuminum) and *Pimpinella anisum* (Anise) prepared by the maceration and soxalet extraction method exhibited an ability to counter the growth of both kinds of bacteria. (preventive and killing). In our study, when the antimicrobial effect was compared between plant species, it was determined that the maceration extract of *Nigella sativa*, which inhibited the microbial activity the most and stopped the microbial activity against *Staphylococcus aureus* and *E. coli* bacteria. *Pimpinella anisum* (Anise) prepared in methanol by the maceration and soxalet extraction method showed more antibacterial effects against *Staphylococcus aureus than E. coli.* In addition, it was determined that the antibacterial effect of the extracts obtained by the maceration method was higher than the extracts obtained by the soxalet extraction method.

In research on the make-up of plants, the crucial active elements found in Nigella sativa are composed of thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethole, sesquiterpene, longifolene, α-pinene, and thymol [10]; The most important active components of *Cuminum cuminum* are alkaloid, anthraquinone, coumarin, flavonoid, glycoside, protein, resin, saponin, tannin and steroid [11]; The most important active components of *Pimpinella anisum* are stated as trans-anetole, estragole, γ-hymachalen, para-anisaldehyde and methyl cavicol [12]. Previous studies have demonstrated that these substances possess antibacterial effects due to the active components in the plants causing damage to the cell wall and leading to cell death [13].

4. Conclusion and Suggestions

This study has an important meaning for the treatment of infections by compare the antibacterial properties of *Nigellla sativa* (black cumin), *Cuminum cuminum* (Cuminum) and *Pimpinella anisum* (Anise) seed extracts prepared in methanol by maceration and soxalet extraction method. All of the seed extracts have a broad spectrum of in vitro activity against *S. aureus* and *E. coli*. Determined antimicrobial activity of plant extracts prepared using different methods and different solvents; suggested that these plants grown in our country may be a source for newly synthesized chemotherapeutics.

Contributions of the authors

Corresponding author: writing (original draft, review & editing), investigation, in vitro studies and interpretation of experimental results.
Coauthor: Methodology, investigation, literature review, in vitro studies, and interpretation of experimental results.

Conflict of Interest Statement: There is no conflict of interest between the authors.
Statement of Research and Publication Ethics: Research and publication ethics compiled within the study.

Conflict of Interest Statement

There is no conflict of interest between the authors

Statement of Research and Publication Ethics

The study is complied with research and publication ethics

References

[1] C. Cordeiro Marina, "Phytochemical and pharmacological investigations of Bridelia retusa Spreng," 2012.

[2] A. G. Fankam, J. R. Kuiate, and V. Kuete, "Antibacterial activities of Beilschmiedia obscura and six other Cameroonian medicinal plants against multi-drug resistant Gram-negative phenotypes," *BMC Complement. Altern. Med*., vol. 14, no. 1, p. 241, 2014.

[3] M. Lahlou, "The success of natural products in drug discovery," *Pharmacol. Pharm.*, vol. 04, no. 03, pp. 17–31, 2013.

[4] M. Saxena , J. Saxena , R. Nema , D. Singh , A. GuptaJ.Eczacılık. fitokimya, 1 (2013)

[5] E. M. Abdallah, "Black seed (Nigella sativa) as antimicrobial drug: A mini-review," *Novel Approaches in Drug Designing & Developmen*t, vol. 3, no. 2, pp. 9–13, 2017.

[6] R. P. Singh, G. H.v., and M. K, "Cuminum cyminum – A popular spice: An updated review," *Pharmacogn. J.*, vol. 9, no. 3, pp. 292–301, 2017.

[7] A. Amer and U. Aly, "Antioxidant and antibacterial properties of anise (Pimpinella anisum L.)," *Egypt. Pharm. J.*, vol. 18, no. 1, p. 68, 2019.

[8] M. Azizi, H. K. Farshchi, F. Oroojalian, and H. Orafaee, "Green synthesis of silver nano-particles using Kelussia odoratissima mozaff. Extract and evaluation of its antibacterial activity," *Journal of Agricultural Science and Technology*, vol. 19, no. 3, pp. 681–691, 2017.

[9] F. Erci, R. Cakir-Koc, and I. Isildak, "Green synthesis of silver nanoparticles using Thymbra spicata L. var. spicata (zahter) aqueous leaf extract and evaluation of their morphology-dependent antibacterial and cytotoxic activity," *Artif. Cells Nanomed. Biotechnol*., vol. 46, no. sup1, pp. 150–158, 2018.

[10] S. Singh, S. S. Das, G. Singh, C. Schuff, M. P. de Lampasona, and C. A. N. Catalán, "Composition, in vitro antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black cumin seeds (Nigella sativa L.)," *Biomed Res. Int*., vol. 2014, p. 918209, 2014.

[11] Q. Chen et al., "In vitro comparison of antioxidant capacity of cumin (Cuminum cyminum L.) oils and their main components," *Lebenson. Wiss. Technol*., vol. 55, no. 2, pp. 632–637, 2014.

[12] A. Shojaii and M. Abdollahi Fard, "Review of Pharmacological Properties and Chemical Constituents of Pimpinella anisum," *ISRN Pharm*., vol. 2012, p. 510795, 2012.

[13] K. O. Saygi, B. Kacmaz, and S. Gul, "Antimicrobial activities of coriander seed essential oil and silver nanoparticles," *Dig. J. Nanomater. Biostructures*, vol. 16, no. 4, pp. 1527-1535, 2021.