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Antibacterial Effects of Methanolic Extract of *Myristica fragrans* against *Klebsiella pneumoniae* and *Acinetobacter baumannii* producing Broad-Spectrum beta-lactamase

Elham Nikouie[1], Ashraf Kariminik[*2]

[1]Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran.
[2]Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran.
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Abstract

The increase in various strains of antibiotic-resistant bacteria have become one of the primary concerns. Therefore, efforts to use plant-derived drugs against drug-resistant bacteria have gained special importance. The aim of this study was to determine the antibacterial effects of methanol extract of *Myristica fragrans* against *Klebsiella pneumoniae* and *Acinetobacter baumannii* isolates producing broad-spectrum beta-lactamases. The plant extract was prepared using the maceration method. Then, the extract was filtered through Whatman filter paper No.1 one and concentrated and dried using a rotary evaporator system. Concentrations of 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 mg/mL of the extract were prepared in dimethyl sulfoxide and methanol (1:1v/v) as solvents. The identification of beta-lactamase-producing isolates was carried out using the phenotype method with antibiotic of cefotaxime and the combination of cefotaxime/clavulanic acid. The antibacterial activity against the isolates was investigated using the agar well diffusion method. Based on the results, 33% of the *Klebsiella* isolates and 50% of the *Acinetobacter* isolates were found to be beta-lactamase producers. All of the isolates were sensitive to the methanol extract of *Myristica fragrans*, with the average minimum inhibitory concentration 10 and 5 mg/mL, respectively. According to the findings, it can be inferred that the *Myristica fragrans* extract has In vitro the ability to *Klebsiella* and *Acinetobacter* isolates. Therefore, with further research and identification of active compounds, it may be possible to use this extract as an alternative antibiotic for treatment in the future.

Key words: Antibacterial activity, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, beta-lactamase, *Myristica fragrans*.

*Corresponding Author: E-mail: a.kariminik@iauk.ac.ir

Introduction

The rapid emergence of antibiotic-resistant bacteria worldwide is concerning and poses a threat to the effectiveness of antibiotics. There is particular concern regarding Gram-negative pathogens as they have developed resistance to almost all available antibiotic options, creating situations reminiscent of the pre-antibiotic era. The most serious Gram-negative infections are usually caused by *Klebsiella pneumoniae* and *Acinetobacter baumannii* (Lee Ventola,2015). Beta-lactam antibiotics are among the most commonly prescribed agents due to their minimal side effects and broad spectrum of antibacterial activity. However, resistance to beta-lactam compounds is a growing problem, and the production of various beta-lactamases is the main reason for this resistance, especially in Gram-negative bacteria (Khalifa et al,2021). *Klebsiella pneumoniae* has become one of the most common Gram-negative pathogenic bacteria due to the emergence of resistance to most antibiotics. Currently, microbial resistance in *Klebsiella pneumoniae* is a significant clinical problem (Wei et al,2018). *Acinetobacter* species have also developed resistance to beta-lactam antibiotics through the production of beta-lactamases. The current drug of choice for treating hospital-acquired infections caused by *Acinetobacter* is carbapenem. However, the number of carbapenem-resistant *Acinetobacter* strains is increasing worldwide (Alkasaby and Zaki,2017).

Indeed, derivatives from medicinal plants can provide new approaches against antibiotic-resistant bacteria. Plant-derived compounds have shown potential as alternative antimicrobial agents and have been investigated for their antibacterial properties. These natural compounds often possess diverse chemical structures and mechanisms of action, which can help overcome bacterial resistance. Research into plant-derived compounds offers promising avenues for the development of novel strategies to combat antibiotic-resistant bacteria (Karpinski et al,2021). *Myristica fragrans*, which belongs to the Myristicaceae family, is a flowering plant that is native to Asia, Africa, the Pacific Islands, and America. Traditionally, different parts of this plant have been used to treat various diseases. Additionally, several scientific reports suggest that this plant exhibits potential antioxidant, antimicrobial, anti-inflammatory, wound-healing, and anticancer activities (Ashokkumar et al,2022). The aim of this study was to determine the antibacterial effects of the methanolic extract of *Myristica fragrans* against strains of *Klebsiella pneumoniae* and *Acinetobacter baumannii* that produce beta lactamase.

Materials and Methods

Preparation of plant extract

The extraction of *Myristica fragrans* seeds was performed using the maceration method and filtered through filter paper No.1. Different concentrations were then prepared in dimethyl sulfoxide (DMSO) and methanol (1:1 v/v) as solvents (Cakupewa et al.,2022).

Isolation and identification of the beta-lactamase producing isolates

In this study, clinical samples were randomly collected from patients using a sterile and moist swab. The swabs were then transferred to tubes containing physiological saline and streaked onto agar plates, including blood agar and MacConkey agar (Merck, Germany). The plates were then incubated at 37 degrees Celsius for 24 hours. After growth, the colonies were subjected to Gram staining, catalase, oxidase, urease, Triple Sugar Iron (TSI), indole, Simon's citrate agar, and motility tests. ESBL (Extended Spectrum Beta-Lactamase) isolates were identified based on antibiotic susceptibility testing and other relevant experiment (Rawy et al,2020). For the identification of strains of beta lactamase-producing *Klebsiella pneumoniae* and *Acinetobacter baumannii*, the combined disk method of cefotaxime/clavulanic acid was used. Bacterial cultures grown for 24 hours were prepared to a concentration equivalent to 0.5 McFarland in sterile normal saline and then uniformly streaked onto Mueller-Hinton agar plates (Merck, Germany). Cefotaxime disks and cefotaxime/clavulanic acid combined disks were placed on the agar surface with a distance of 20 mm between them. Isolates that showed an inhibition zone diameter around the combined

disk of more than 5 mm compared to the inhibition zone diameter around the individual disk were considered as beta lactamase-producing or extended-spectrum beta-lactamase (ESBL)-producing bacteria (Dhara et al.,2020).

Antibacterial investigation

The agar well diffusion method was employed to investigate the antibacterial effects of the methanolic extract from *Myristica fragrans* seeds. In this method, wells are created in agar plates, and the extract is then placed in these wells. Extracts at concentrations of 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 mg/mL prepared in dimethyl sulfoxide and methanol (1:1 v/v). The extract then diffuses into the surrounding agar, and after the incubation period, antibacterial activity can be observed by measuring the zone of inhibition around the wells, indicating the inhibition of bacterial growth. The minimum inhibitory concentration (MIC) is determined. Dimethyl sulfoxide and methanol (1:1 v/v) were used as the negative control (Hassan and Ullah,2019; Shahabinejad and Kariminik, 2019).

Results

In this study, 60 isolates of *K. pneumoniae* and 40 isolates of *A. baumannii* were identified from clinical samples. *K. pneumoniae* was identified by mucoid and rod-shaped colonies, Gram-negative, oxidase-negative, indole-negative, non-motile, urease-positive, citrate-positive, and fermentation of glucose, lactose, and sucrose. *A. baumannii* was identified by the Gram-negative, rod-shaped colonies, positive catalase, negative oxidase, and inability to ferment sugars. The ESBL-producing isolates were identified based on the results of cefotaxime and cefotaxime/clavulanic acid combined disk antibiotic susceptibility testing. Among the *Klebsiella* isolates, 33% (20 isolates) were ESBL producers, and among the *Acinetobacter* isolates, 50% (20 isolates) were also ESBL producers. The effect of the methanolic extract of *Myristica fragrans* seed at different concentrations on 20 isolates of *K. pneumoniae* and 20 isolates of ESBL -producing *A. baumannii* is shown in Tables 1 and 2 and Figures 1 and 2, respectively. The findings showed that a total of 50% of the investigated isolates were ESBL producers. Additionally, the methanolic extract of *Myristica fragrans* seeds at concentrations of 10, 20, 40, and 80 mg/mL was effective against ESBL-producing *Klebsiella* bacteria, while at concentrations of 80, 40, 20, 10, and 5 mg/mL, it was effective against ESBL-producing *Acinetobacter* bacteria. The average minimum inhibitory concentration (MIC) for growth inhibition was determined to be 10 mg/mL for *K. pneumoniae* and 5 mg/mL for *A. baumannii*. Based on the obtained results, it can be concluded that the methanolic extract of *Myristica fragrans* seed at low concentrations has highly desirable antibacterial effects against the investigated strains that are resistant to beta-lactam antibiotics. Therefore, it can be inferred that by identifying the effective and pure compounds present in this plant, suitable antibacterial agents can be developed and produced against certain Gram-positive and Gram-negative bacteria, especially the bacteria studied in this research.

Discussion

The increasing prevalence of antibiotic resistance has led to significant challenges in the treatment of infections, particularly hospital-acquired infections. For instance, based on conducted research, *K. pneumoniae* is an opportunistic pathogen commonly found in hospital intensive care units and has developed resistance to antibiotics. Beta-lactam antibiotics, including the important family of carbapenems, are among the primary treatment options, but *Klebsiella pneumoniae* produces beta-lactamase enzymes to counteract them. The indiscriminate use of these antibiotics has led to the emergence of another group of beta-lactamase enzymes called ESBL, which have a broad spectrum of activity (Ahanjan,2014). In addition, extensive studies have been conducted on the emergence of various drug resistance mechanisms in *Acinetobacter* species, with *A. baumannii* being the predominant species in most cases. *Acinetobacter* species are considered one of the most successful pathogens in modern healthcare systems, and their prevalence is influenced by various factors such as surgical procedures, the use of different antibiotics, and the number of immunocompromised patients. The unique capabilities of

Table 1. Antibacterial effects of the methanolic extract of *Myristica fragrans* seeds on ESBL-producing *K. pneumoniae* by the well diffusion method, Numbers: Inhibition zone (mm).

Concentration (mg/mL) / Isolates	80	40	20	10	5	2.5	1.25
1	18	11	10	10	-	-	-
2	12	12	11	9	-	-	-
3	17	15	12	10	-	-	-
4	14	13	11	8	-	-	-
5	16	14	12	10	-	-	-
6	15	14	12	11	-	-	-
7	16	15	13	11	-	-	-
8	18	18	11	9	-	-	-
9	14	13	12	11	-	-	-
10	13	12	11	-	-	-	-
11	15	14	11	8	-	-	-
12	16	15	12	10	-	-	-
13	18	17	16	11	-	-	-
14	15	13	12	10	-	-	-
15	16	14	12	9	-	-	-
16	17	17	14	11	-	-	-
17	12	11	10	-	-	-	-
18	17	15	13	10	-	-	-
19	13	10	8	-	-	-	-
20	15	13	12	11	-	-	-

Table 1. Antibacterial effects of the methanolic extract of *Myristica fragrans* seeds on ESBL-producing *A. baumannii* by the well diffusion method, Numbers: Inhibition zone (mm).

Concentration (mg/mL) / Isolates	80	40	20	10	5	2.5	1.25
1	36	30	26	20	19	-	_
2	22	22	20	16	-	-	_
3	31	28	24	20	17	-	_
4	12	10	8	8	-	-	_
5	28	24	21	20	18	-	_
6	15	14	11	11	-	-	_
7	28	25	23	21	17	-	_
8	18	18	12	10	-	-	_
9	24	23	18	12	-	-	_
10	33	30	26	21	18	-	_
11	15	14	13	8	-	-	_
12	26	25	22	18	17	-	_
13	18	15	14	11	-	-	_
14	25	23	19	17	-	-	_
15	18	18	16	12	-	-	_
16	28	27	24	21	17	-	_
17	23	23	21	18	-	-	_
18	17	14	12	10	-	-	_
19	23	20	18	12	-	-	_
20	25	23	22	18	18	-	_

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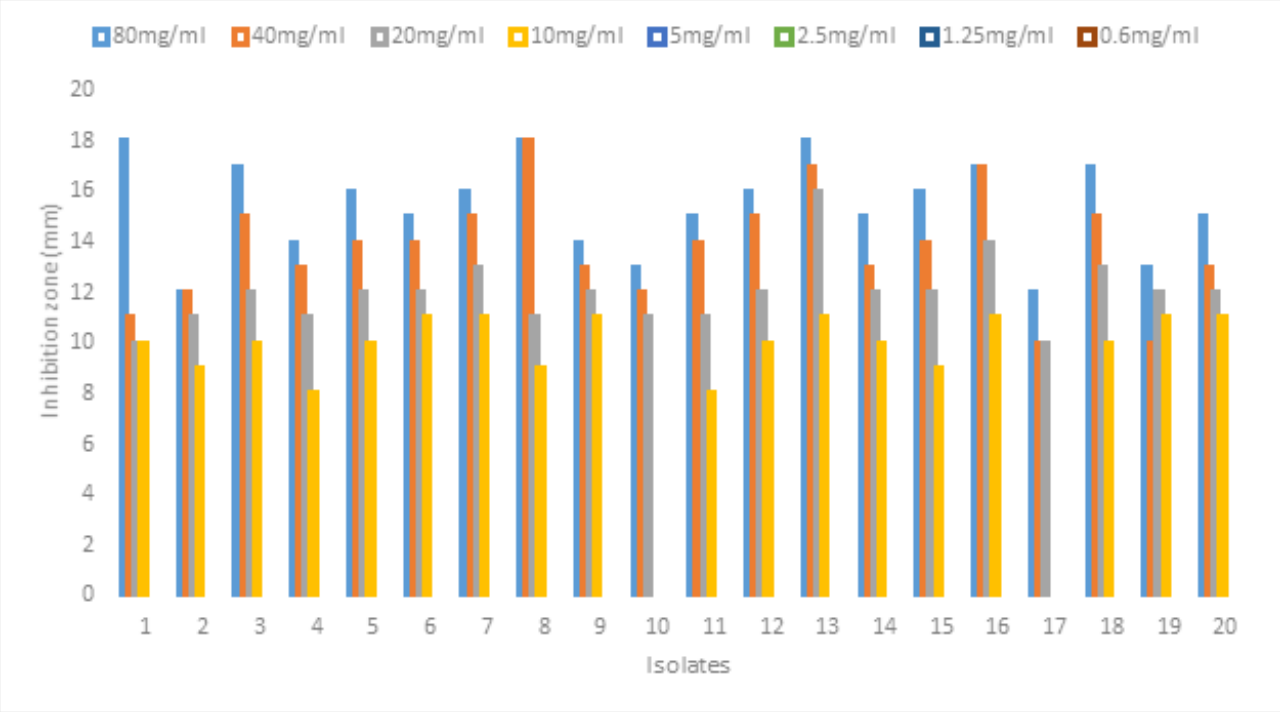

Figure 1. Dose response of *Myristica fragrans* extract against 20 ESBL-producing *K. pneumoniae* isolates

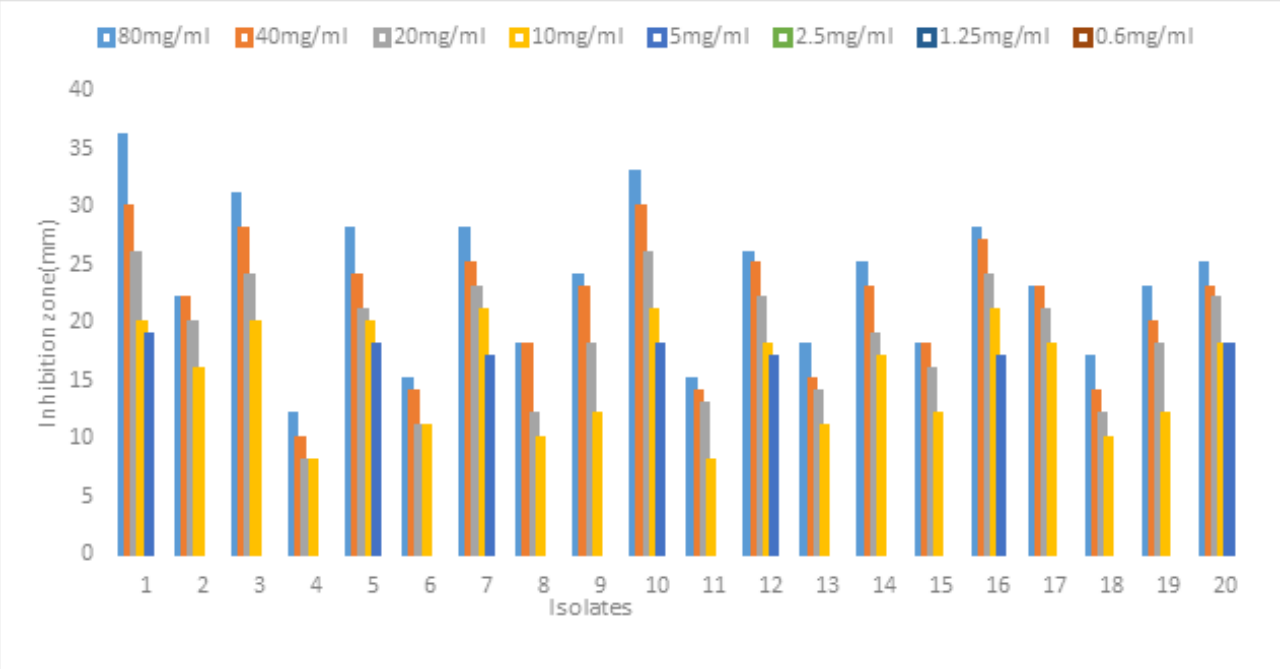

Figure 1. Dose response of *Myristica fragrans* extract against 20 ESBL-producing *A. baumannii* isolates

this bacterium, which contribute to the production of resistance factors and its widespread dissemination worldwide, have garnered significant attention from researchers (Mahmudpour et al,2019). In line with the present study, researchers analyzed 54 chemical compounds of *Myristica fragrans* oil using gas chromatography-mass spectrometry (GC-MS). *Myristica fragrans* oil exhibited significant antibacterial activity. It showed a notable effect in inhibiting the growth of *Escherichia coli*, and the results of this research indicate that the antibacterial mechanism of *Myristica fragrans* oil against bacteria may be attributed to disturbances in cell membrane integrity and inhibition of DNA synthesis (Cui et al,2015). In another study, the antioxidant and antimicrobial activity of *Myristica fragrans* essential oil was investigated. Twenty-five compounds were identified in this oil, with the most abundant compound being the monoterpene hydrocarbon sabinene. The antimicrobial activity of the *Myristica fragrans* essential oil was tested using the disc diffusion method against Gram-negative bacteria, including *Escherichia coli*, *K. pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*. The tested Gram-negative bacteria showed a brief sensitivity to the *Myristica fragrans* essential oil (Nikolic et al,2022).

Conclusion

The current study presents a promising solution to combat antimicrobial resistance through the utilization of a natural herbal extract. The extract derived from *Myristica fragrans* exhibits potent antibacterial activity, effectively inhibiting or even halting the growth of drug-resistant microbial populations belonging to various bacterial strains. This suggests that the extract could be utilized as a food additive or preservative to control the growth of these microbial populations, thereby safeguarding the health of both humans and animals.

Conflicted of Interest

No conflicts of interest

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