

# Antimicrobial Activity of Seeds and Leaves of *Myristica fragrans* against Multi-resistant Microorganisms

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**Abstract:** *Myristica fragrans*, known as nutmeg, is used in food applications. In traditional medicines, the seeds and leaves are used to treat skin, respiratory and gastrointestinal diseases. Limited antibacterial activity has been reported for this plant. The present study aimed to screen the decoction and methanolic extracts of the seeds and leaves of *M. fragrans* against *Staphylococcus aureus* NCTC 6571, five strains of methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa* NCTC 10662, *Escherichia coli* NCTC 10418, as well as essential oils from both parts against a panel of resistant bacteria and *Candida* spp. in order to identify potential antimicrobial activity. Antimicrobial activity was evaluated using well (well diameter: 12 mm) and disc diffusion methods (disc diameter: 6 mm). The decoction and methanolic extract of leaves and methanolic extract of seeds of *M. fragrans* showed inhibitory activity against *S. aureus* and all five MRSA strains with zones of inhibition (ZOI) = 16.0 ± 0.0 mm to 19.0 ± 0.0 mm. The decoction and methanolic extract of both parts did not show inhibitory activity against *E. coli* and *P. aeruginosa*. However, both essential oils showed inhibitory activity against *S. aureus* and the five MRSA strains, as well as *E. coli* (ZOI = 9-15 mm). The essential oils from seed showed activity against all tested multi-resistant bacteria (ZOI = 7-12 mm). The essential oils from leaves showed activity against *Klebsiella pneumoniae*, *Acinetobacter* spp., *Enterobacter cloacae* and group A beta-haemolytic streptococcus (ZOI = 8-12 mm). The essential oils showed inhibitory activity against all tested *Candida* species (ZOI = 8-15 mm). The decoction, methanolic extract and essential oils of leaves have potential activity against sensitive and resistant *S. aureus*. This is the first report of inhibition of multi-resistant bacteria and *Candida* spp. by the essential oils of leaves and seeds of *M. fragrans* which could be utilized for pharmaceutical applications.

**Key words:** Antimicrobial activity, *Myristica fragrans*, multi-resistant microorganisms.

## 1. Introduction

The indigenous medical system of Sri Lanka consists of Ayurveda, Siddha and Unani systems of medicine. About 1,500 species of medicinal plants are used to prepare traditional medicines in Sri Lanka [1]. Currently, research is focused on investigation of these medicinal plants and herbal drugs for antimicrobial, antidiabetic, gastroprotective, lipid lowering and anticarcinogenic activities [2]. Plants synthesize secondary metabolites, which play a major role to protect the plants from environmental hazards, including microbes, predators and climatic conditions. These compounds, responsible for bioactivity, are

being screened to identify the active components with the potential of developing new classes of drugs for common diseases [2].

Infectious diseases are the second leading cause of death in the world. It is reported that 16.2% of people die due to infection each year [3]. Resistance to antibiotics is increasing and is of concern worldwide. Hence, screening of medicinal plants, followed by active compound isolation for possible formulation of new drugs, is needed.

*Myristica fragrans* is a well known spice (Family Myristicaceae; Sinhala: Jathikka; Tamil: Sathikkai; English: Nutmeg) [4] with leaves which have an aromatic odour when crushed. Nutmeg is the seed of the tree, roughly egg-shaped and usually used in

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powdered form. Several other commercial products, such as essential oils, extracted oleoresin and nutmeg butter, are also produced from this tree. In traditional medicines, the leaves and seeds are one of the ingredients, especially in Siddha medicines, such as Parankikilangu choornam, Periyapatpam, Vellaruku patpam, Astabirava kulikai, Thankaellathi mathirai, Kakkuvan lehiyam, Impooral lehiyam, Karisalai lehiyam and Brinhamila thylam [5]. These Siddha medicines are used for the treatment of skin, respiratory and gastrointestinal diseases [5]. Antibacterial activity of water, ethanol and acetone extracts of the seeds of *M. fragrans* have been previously tested against two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) [6]. The aim of the study was to screen the antibacterial activity of the decoction and methanolic extracts of *M. fragrans* against 15 bacterial isolates and eight *Candida* spp. obtained from the Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka.

## 2. Materials and Methodology

### 2.1 Plant Collection

Fresh leaves and dried seeds of the plant were collected from Kandy during September to October, 2011. These were identified and authenticated at the National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. The leaves were washed, dried under shade, coarse powdered and packed in polythene bags to prepare the decoction and extracts.

#### 2.1.1 Preparation of Decoction

Coarse powdered leaves and seeds of *M. fragrans* (40 g each) were taken separately, distilled water added (480 mL) and boiled until the volume was reduced to 60 mL (1/8), and further concentrated to obtain 30 mL using a reduced flame.

#### 2.1.2 Preparation of Methanolic Extract

Leaves and seeds (100 g each) of the plant were extracted with methanol at 65 °C for 6 h using the

Soxhlet extractor.

#### 2.1.3 Distillation of Essential Oil

Leaves (100 g) and seeds (50 g) of *M. fragrans* were hydrodistilled at 100 °C for 8 h using the Clevenger apparatus to obtain the essential oils. The volume of oils was measured using the scaled Clevenger arm. The yield (mL) was calculated as mL/100 g based on the dry weight of sample.

### 2.2 Test Microorganisms

The plant extracts were assayed for antibacterial and antifungal activity against 15 bacterial isolates and eight *Candida* spp. obtained from the Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka.

The organisms were divided into three groups according to their inherent susceptibility patterns. Panel 1 consisted of three control strains (*S. aureus* NCTC 6571, *E. coli* NCTC 10418, *P. aeruginosa* NCTC 10662) and five methicillin-resistant *S. aureus* (MRSA) strains; panel 2 consisted of the multi-drug resistant (MDR) bacteria *K. pneumoniae*, extended spectrum  $\beta$ -lactamase (ESBL) producing *K. pneumoniae*, *Acinetobacter* spp., *E. cloacae*, *Proteus* spp., vancomycin-resistant *Enterococcus* sp. (VRE) and group A beta-haemolytic streptococcus (BHS); and panel 3, eight *Candida* spp. (*C. tropicalis* ATCC 13803, *C. krusei* ATCC 6258, *C. albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. parapsilosis* ATCC 22019 and three clinical isolates of *C. albicans*).

### 2.3 Antibacterial Assay

The antibacterial activity was evaluated using the well diffusion method for decoction and methanolic extracts. Disc diffusion method was used to detect the activity of essential oils of leaves and seeds of *M. fragrans*. All the experiments were conducted in triplicate using standard aseptic techniques. The bacterial isolates were maintained on nutrient agar slopes at room temperature. Each isolate was

subcultured on blood agar and checked for purity before use.

### 2.3.1 Preparation of Bacterial Inocula (0.5 MacFarland Standard)

Each isolated bacterial colony was taken separately onto a sterile cotton wool plug and smeared on the inner wall of a sterile universal bottle containing approximately 2 mL of sterile normal saline. The bottle was capped and vortexed for 5 s to uniformly suspend the bacterial culture. The turbidity of the suspension was made similar to that of the 0.5 MacFarland standard by the addition of more microorganisms or dilution with more normal saline.

### 2.3.2 Well Diffusion Method

Mueller-Hinton agar (MHA) was used for this bioassay. The MHA plate was inoculated with 1 mL of the bacterial suspension and Petri dish rotated to ensure uniform spread. Excess liquid was removed from the plate which was allowed to dry at 37 °C for 15 min. Wells 12 mm in diameter and 4 mm in depth were bored into the MHA using a sterile cork borer and completely filled with the test extract (decoctions and methanolic extract) and methanol only as control. The plates were left on the bench for 30 min for absorption of the extract and incubated at 37 °C for 24 h. The plates were examined for inhibition of growth

around the well and diameters of inhibition zone (ZOI) were measured.

### 2.3.3 Disc Diffusion Method

Blank paper discs (6 mm diameter) were used to detect the activity of essential oils of leaves and seeds of *M. fragrans*. The procedure was the same as for the well diffusion method with paper discs replacing the wells. MHA was used as media for bacteria and Sabourad dextrose agar (SDA) was used as media for *Candida* spp..

## 3. Results

The yield percentage of methanolic extracts and essential oils of leaves and seeds are given in Table 1. The seed yielded higher percentage of essential oil (14 mL/100 g) compared to leaves (2 mL/100 g).

The ZOI of decoction and methanolic extracts of leaves and seeds of *M. fragrans* are given in Table 2. Decoction and methanolic extract of leaves and methanolic extract of seeds of *M. fragrans* showed inhibitory activity against *S. aureus* and all five MRSA strains. Diameter of ZOI ranged from 16.0 ± 0.0 mm to 19.0 ± 0.0 mm. Decoction of seeds of *M. fragrans* did not show activity against all tested microorganisms. *E. coli* and *P. aeruginosa* were not inhibited by any tested extracts.

**Table 1 Yield of methanolic extract and essential oils of leaves and seeds.**

Plants	Methanolic extract (g/100 g)	Essential oil (mL/100 g)
Seed of <i>M. fragrans</i>	21.44	14
Leaf of <i>M. fragrans</i>	23.02	2

**Table 2 Diameter of ZOI (mm) of decoction and methanolic extract of leaves and seeds of *M. fragrans* using the well diffusion method.**

Microorganisms	Decoction of <i>M. fragrans</i>		Methanolic extract of <i>M. fragrans</i>	
	Leaf	Seed	Leaf	Seed
<i>S. aureus</i> NCTC 6571	18.6 ± 0.5	-	18.0 ± 0.0	17.5 ± 0.4
<i>E. coli</i> NCTC 10418	-	-	-	-
<i>P. aeruginosa</i> NCTC 10662	-	-	-	-
MRSA strain 1	18.0 ± 0.8	-	18.0 ± 0.0	17.3 ± 0.4
MRSA strain 2	17.0 ± 0.0	-	19.0 ± 0.0	17.0 ± 0.0
MRSA strain 3	17.3 ± 0.2	-	19.0 ± 0.0	17.3 ± 0.4
MRSA strain 4	17.0 ± 0.2	-	19.0 ± 0.0	17.0 ± 0.0
MRSA strain 5	16.0 ± 0.0	-	19.0 ± 0.0	17.5 ± 0.4

Data represented as mean ± standard deviation for methanolic and water extracts (*n* = 4 each).

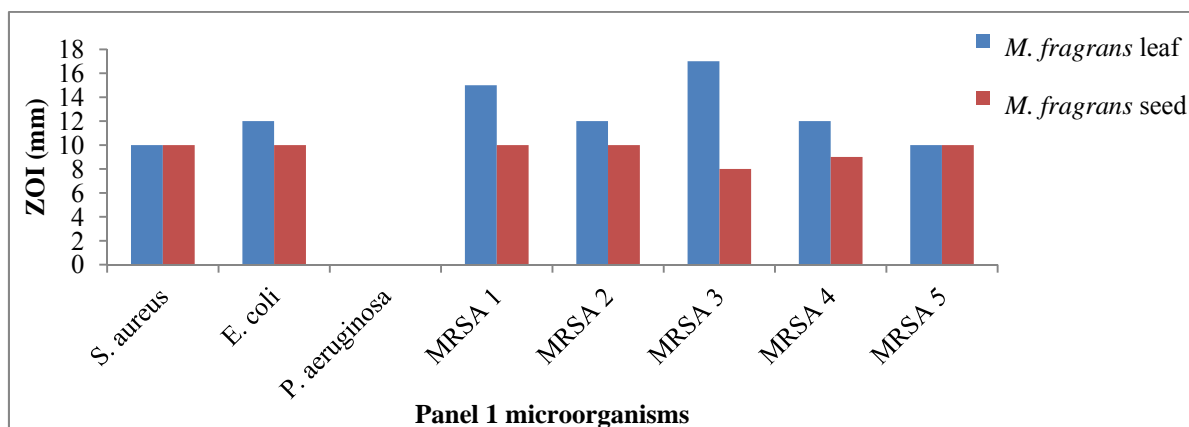


Fig. 1 Antibacterial activity (diameter of ZOI in mm) of essential oils of *M. fragrans* seeds and leaves using disc diffusion method against panel 1 microorganisms.

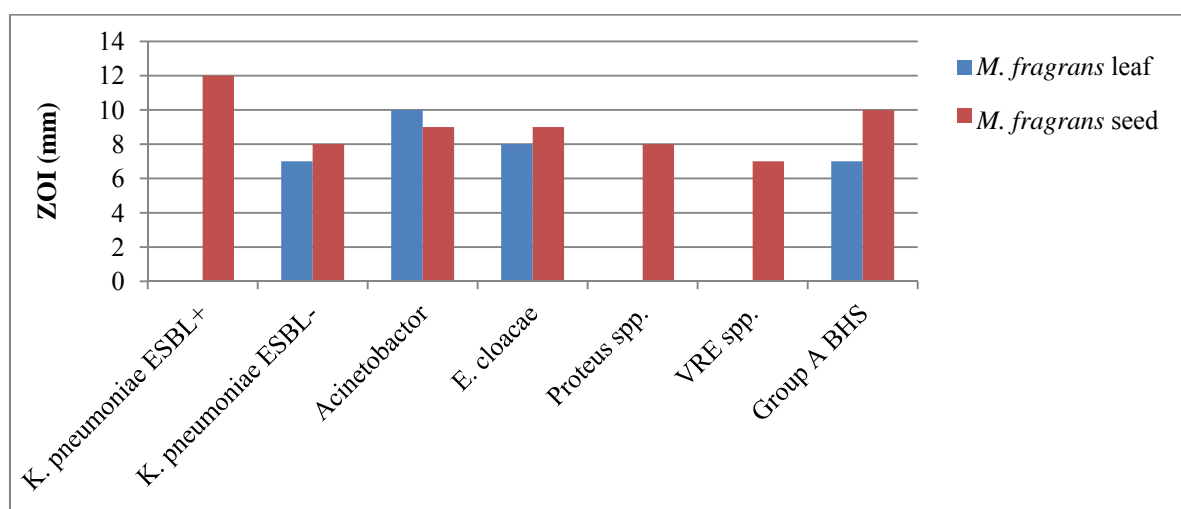


Fig. 2 Antibacterial activity of oils of seeds and leaves of *M. fragrans* against panel 2 organisms (multi-resistant microorganisms).

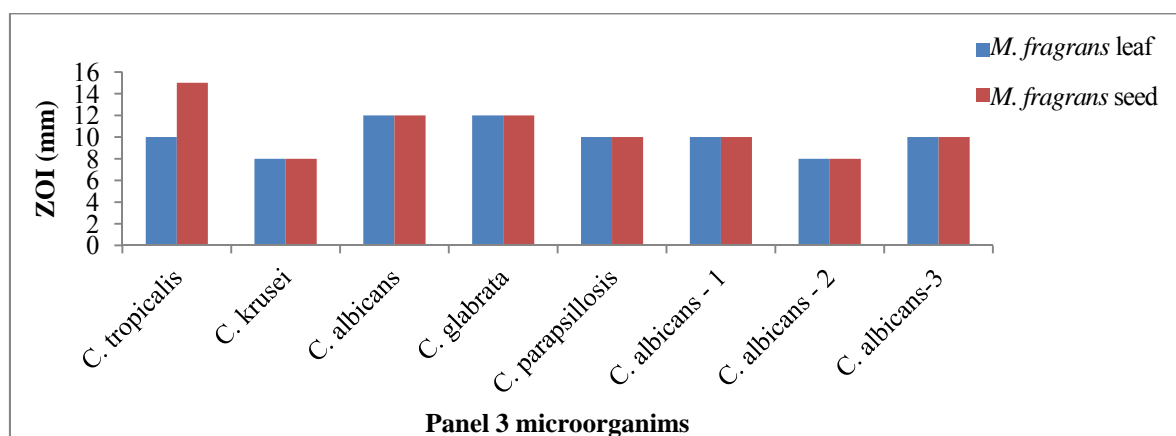


Fig. 3 Antibacterial activity of oils of seeds and leaves of *M. fragrans* against panel 3 microorganisms (*Candida* spp.).

The antimicrobial activity of essential oils of leaves and seeds of *M. fragrans* screened against the three groups of organisms (panels 1-3) are shown in

Figs.1-3, respectively. The essential oils of leaves and seeds of *M. fragrans* showed inhibitory activity against *S. aureus*, *E. coli* and all tested five MRSA

strains, while did not show activity against *P. aeruginosa*. The essential oil of seed of *M. fragrans* showed activity against all tested multi-resistant bacteria (ZOI = 7-12 mm), but the essential oil of leaves, however, only showed activity against *K. pneumoniae*, *Acinetobacter* spp., *E. cloacae* and group A BHS. The essential oils of leaves and seeds of *M. fragrans* showed inhibitory activity against all tested *Candida* spp.. The ZOI diameter of essential oil from leaves was similar to that of essential oil from seed, except against *C. tropicalis*.

#### 4. Discussion

##### 4.1 Decoction and Methanolic Extracts of Seeds and Leaves of *M. fragrans*

In traditional medicine, both fresh and dried plant material are used in the treatment as different forms, such as decoction, choornam and tablet. In this current study, the dried seeds and leaves of *M. fragrans* were used to prepare extracts. Water and methanol were used as solvents to extract the compounds of seeds and leaves.

The decoction of seeds of *M. fragrans* did not show activity against all tested microorganisms. The methanolic extract, in contrast, showed activity against sensitive and resistant strains of *S. aureus*. Plants extracted in an organic solvent (methanol) provide more consistent antimicrobial activity compared to aqueous extracts of the same plants [7]. Most antimicrobial active compounds from plant origin that have been identified were soluble in polar solvents, such as methanol and ethanol, instead of water [8]. A decoction is prepared with water which is a polar solvent. Methanol is also a polar solvent. In ancient times, there was no scientific (technological) method to separate bioactive compounds and water was primarily used as the solvent. Methanol has a polarity index of 5.1 and is used for extraction of various polar compounds. However, certain groups of non polar compounds are less soluble in methanol. Extraction techniques are also important to separate

the active substances, because some active compounds may be destroyed by heat [9]. Methanol has a low boiling point (65 °C). The efficiency of extraction of bioactive compounds from plant materials using the soxhlet apparatus is high. In addition, the extracts need not to be filtered and the removal of solvent from the concentrated extract using the rotary evaporator is easy. In the current study, these techniques were used to extract phytochemicals from seeds and leaves of *M. fragrans*. The decoction of seed did not show activity, even though the decoction of leaves showed activity. It may be due to some additional compounds present in the leaves.

The decoction and methanolic extract of leaves of *M. fragrans* showed inhibitory activity against *S. aureus* and all five MRSA strains. Diameters of ZOI of these extracts are almost the same ( $16.0 \pm 0.0$  mm to  $19.0 \pm 0.0$  mm). *E. coli* and *P. aeruginosa* were not inhibited by any tested extracts (decoction and methanolic). In the current study, the methanolic extract of both seeds and leaves showed activity against *S. aureus*, including sensitive and resistant strains. The antibacterial activity of water, ethanol and acetone extracts of *M. fragrans* seed has been demonstrated previously against methicillin sensitive *S. aureus* [10]. However, in the current study, antibacterial activity of water extracts of the seed was not demonstrable, though similar activity was shown by the methanol extract against *S. aureus*, including MRSA strains. Water, ethanol and acetone extracts of *M. fragrans* seeds tested previously did not inhibit *E. coli* and *P. aeruginosa* [10], which is similar to the results of the present study.

Plant extracts in general have good activity against Gram-positive bacteria, because these bacteria contains only peptidoglycan layer, which is easily penetrated by the antimicrobial compounds in the plant extracts. Gram-negative bacteria contain a single layer of peptidoglycan surrounded by an outer membrane and it is possible that plant extracts are ineffective because they are unable to penetrate the

cell wall of Gram-negative bacteria.

#### 4.2 Essential Oils of Seeds and Leaves of *M. fragrans*

Essential oils are a complex mixture of the volatile organic components of fragrant plant matter that contribute to the flavour and fragrance of the plant. The antimicrobial activity of oil of leaves of *M. fragrans* against *P. vulgaris* and *K. pneumoniae* [11], and seed oil against clinical isolates of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris*, *P. aeruginosa*, *S. aureus* [12] and 25 different species of bacteria, including *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris* and *A. calcoaceticus* [13] have been reported previously. No inhibitory activity was reported against *K. pneumoniae* [11] and *P. aeruginosa* [13]. Previous results showed that the minimum inhibitory concentration (MIC) of oil of seed was 1 mg/mL for *E. coli*, *P. mirabilis* and *P. aeruginosa*, and > 1 mg/mL for *P. vulgaris* and *K. pneumoniae* [12]. In the present study, a wider spectrum of microorganisms, including MRSA as well as multi-resistant *K. pneumoniae* ESBL<sup>±</sup>- producing strains, MDR *E. cloacae*, *Proteus* spp., and VRE were tested. The seed oil of *M. fragrans* showed activity against all tested organisms including the MDR Gram-negative bacilli. In contrast, the oil of leaves did not show activity against *K. pneumoniae*, *Proteus* spp. and VRE. Activity of the seed oil against *Acinetobacter* spp., which is often multi-resistant and causes infections in patients with compromised host defenses, is worth noting, as the current antibiotic armamentarium is often insufficient for treatment of serious infections caused by this species [13].

The antimicrobial activity of oil of leaves of *M. fragrans* has been reported previously against fungi, such as *C. tropicalis*, *C. albicans* and *C. glabrata* [11]. In the current study, in addition to these three species, activity was demonstrated against *C. krusei* and *C. parapsilosis*. Inhibition was demonstrated against all tested *Candida* spp., by both seed and leaf oils.

MDR organisms are resistant to several classes of

antimicrobial agents. MDR is considered as one of the most important problems faced by the healthcare sector at the moment and the discovery of compounds active against these microorganisms are of current importance [3]. Although MRSA and VRE are defined by resistance to a single class of antibiotics, these pathogens are also frequently resistant to other classes of antibiotics [14]. ESBL producers and MDR *Acinetobacter* spp. are increasing in the community and healthcare settings, respectively [15]. The current study showed that inhibitory activity of the tested oils against these microorganisms, and the elucidation of the active components and the mechanism of actions behind these activities is the logical next step in the process of discovering novel anti-bacterial compounds from these natural products.

## 5. Conclusions

The decoction, methanolic extract and essential oil of leaves of *M. fragrans* all have potential activity against sensitive and resistant *S. aureus*. The essential oils of leaves and seeds have the ability to inhibit multi-resistant microorganisms and *Candida* spp. and can be further studied for potential pharmaceutical applications.

## Acknowledgments

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