

Original Research Article

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Antimicrobial Susceptibility of *Myristica fragrans* Extract against Oral Pathogens

Abhilasha Singh^{1*}, Ritu Thakur Bais² and Vinod Singh²

¹Department of Microbiology, Barkatullah University, Bhopal, India

²Department of Botany, Govt. M.L.B. College, Bhopal, India

*Corresponding author:

ABSTRACT

Keywords

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This study was designed to evaluate the antibacterial activity of methanolic *Myristica fragrans* seed extract tested against four bacterial species isolated as oral pathogen. The susceptibility of these different bacterial species toward the extract of this plant seed was compared with each other. Results showed that methanolic extract exhibited antibacterial activity against gram positive bacterial species, the diameter of inhibition zone reached 28 ± 0.14 mm against *E. faecalis* and 24 ± 0.002 mm against *S. mitis* by extract. It is concluded that this plant can be indispensable source for secondary metabolites.

Introduction

Myristica fragrans commonly known as Jaiphal and Javitri in India, belongs to the family Myristicaceae. It produces spice, nutmeg. This spice is used in Asian folk medicine to treat microbial pathogens. Depending on its origins, It contains the aroma compounds as like monoterpenes (87.5%), monoterpene alcohols (5.5%), and other aromatics (7%) (Pooja *et al.*, 2012). The oil of nutmeg has also been used for medicinal purposes and it is this fraction of the nutmeg which contains the pharmacologically active components (Kaushik and Singh, 2012). Narasimhan and Dhake, 2006 reported that trimyristin, an

active compound obtained from seed of *Myristica fragrans*, also exhibited good antibacterial properties against Gram-positive and Gram negative bacteria. The great diversity of the chemical structures of secondary plant metabolites continues to provide new and important leads against several pharmacological targets (Porto, 2009). Several studies have demonstrated the great importance of natural products, both plant extracts and isolated compounds, as natural antibacterial agents in oral care products (Haffajee *et al.*, 2008). Dental caries is a localized infectious disease that leads to loss of teeth. Individuals heavily colonized by

cariogenic bacteria are considered to be at high risk for dental carie (Deshpande and Kadam, 2012). Caries and periodontal disease can be prevented by good maintenance of oral hygiene with the use of oral care products. Nowadays, there are high interests in oral care products that are incorporated with medicinal plant extracts and are used extensively by the consumers due to low toxicity compared to oral care products (Shafiei *et al.*, 2012). The current study investigates the anti-microbial potential of *Myristica fragrans* against oral pathogens.

Materials and Methods

Plant material: Fresh and dry fruits of *M. fragrans* were collected from local market of Bhopal, India. The dry fruits were first identified by Dr. Manish Mishra, Indian Institute of Forest Management, Bhopal, India. The dried fruits were pulverized to a fine powder using a sterilized mixer grinder and stored in airtight polybags. Methanol was used for extraction.

Extraction of Bioactive Compound

The pulverized samples of *Myristica fragrans* were extracted using Soxhlet extraction (Borosil) method with methanol. The extraction processes were carried out for 3 days to obtain crude extracts which were concentrated under reduced pressure by using a rotary evaporator. The resulting pellet was finally pounded to dryness at room temperature to concentrate crude extracts, respectively. The crude extract was then stored at -20°C for use in further studies.

Isolation of Oral Pathogens

The dental plaque sample was inoculated on blood agar, MRS and Macconky plates and incubated for 18-24 hours at 37°C streak plate technique and the pathogens were isolated

and identified by Bergey's manual (Holt *et al.*, 2000).

Statistical Analysis: The data from zones of inhibition of growth of each microorganism in different extracts concentrations were represented as mean \pm standard error.

Results and Discussion

After the bacteria were grown on Blood Agar plate, Macconky agar and MRS agar colonies with distinctive morphology were selected. Four colonies were selected for further study. All of the strains displayed positive gram staining reaction. Different biochemical tests for the presumptive identification of the isolated colonies were also performed. All of the strains were catalase negative. However, they exhibited differential hemolysis pattern on blood agar and distinctive reaction pattern for bile aesculin, bacitracin resistance, arginine hydrolysis and esculin hydrolysis tests.

The most frequently isolated species are *Lactobacillus acidophilus*, *Streptococcus mutans*, *Streptococcus mitis* and *Enterococcus faecalis*. The methanolic extract of *M. fragrans* subjected to preliminary screening for their antibacterial activity against four isolated bacterial species namely *Lactobacillus acidophilus*, *Streptococcus mutans*, *Streptococcus mitis* and *Enterococcus faecalis*. Methanolic extract showed high activity against gram positive. The diameter of inhibition zone reached 28 ± 0.14 mm against *E. faecalis* concentration of 25 mg/ml and 24 ± 0.002 mm for *Streptococcus mitis* at 50 mg/ml concentration (Table 1 & Figure 1&2). Many research studies are available which showed the prevention of dental plaque formation and on different compounds which possess antibacterial activity against oral bacteria (Sujatha *et al.*, 2012).

Table.1 Zone of inhibition of *M. fragrans* extract against different oral pathogens

Test Microorganism	Mean diameter of zone of Inhibition (mm)			
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
<i>Lactobacillus acidophilus</i>	16±0.002	19±0.04	16±0.028	15±0.14
<i>Streptococcus mutans</i>	15±0.04	16±0.04	17±0.002	16±0.04
<i>Streptococcus mitis</i>	16±0.007	24±0.002	11±0.002	13±0.007
<i>Entrococcus faecalis</i>	28±0.14	15±0.007	13±0.002	19±0.007

Fig.1 Antimicrobial activity of *M. fragrans* against oral pathogens

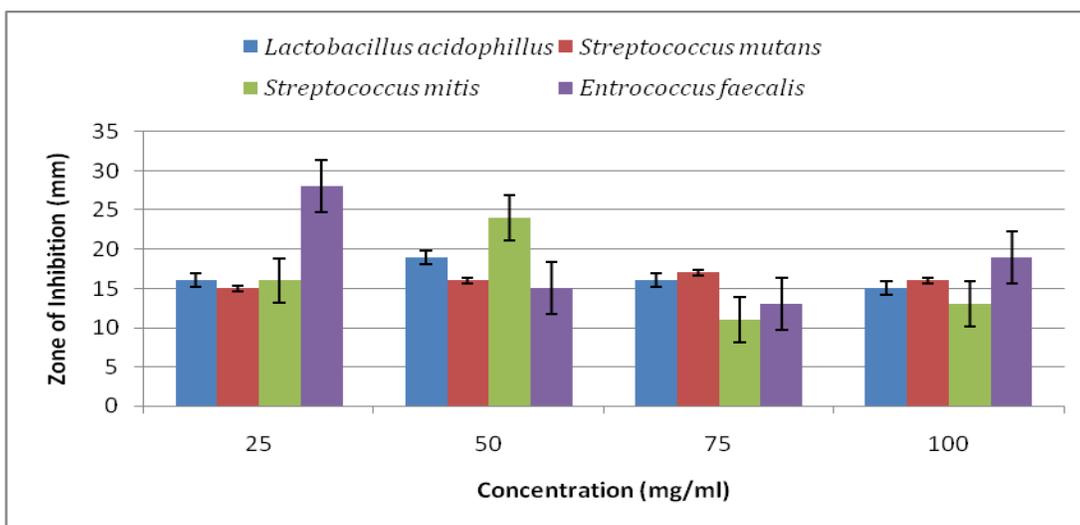


Fig.2 Antimicrobial activity of *M. fragrans* against oral pathogens (A) *L. acidophilus* (B) *S. mitis* (C) *E. faecalis* (D) *S. mutans*





The results obtained from these study support the antimicrobial activity in the previous reports by Piaru *et al.*, 2012. Nutmeg oil contains monoterpenes such as -pinene, camphene, -pinene, sabinene, myrcene, a-phellandrene, a-terpinene, limonene, 1, 8-cineole, g-terpinene, linalool, terpinen-4-ol, safrole, methyl eugenol and myristicin, as their active principles. Their mode of antimicrobial action is related to their ability to inactivate microbial adhesion, enzymes and cell envelope proteins (Ibrahim, *et al.*, 2013). The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. Over the past few years, medicinal plants have regained a wide recognition due to an escalating faith in herbal medicine. In view of its lesser side effects compared to allopathic medicine in addition, the necessity of meeting the requirements of medicine for an increasing human population (Akrayi, 2012).

In conclusion, it can be concluded from the results that methanol extract of *M. fragrans* possess significant antibacterial activity against tested oral bacteria. However, the active components responsible for the antimicrobial activities need to be evaluated further. The antibacterial that are currently

used suffer from several drawbacks such as high toxicity, low absorption, and high cost of treatment. The area of antibacterial drug discovery is at a nascent stage. The challenge in antibacterial drug discovery is its high toxicity in humans. Therefore the search for alternate treatment modalities is the need of the hour.

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