Research Article

Myristica Fragrans: A Potent Antibacterial Agent against Food Borne Pathogens

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ABSTRACT

The global challenge of drug resistance in pathogenic bacteria has intensified due to the excessive use of antibiotics. In context to this, the antimicrobial activity of flower extract of *Myristica fragrans* was evaluated against food borne pathogens including *Salmonella typhimurium*, *Shigella flexneri*, Vibrio cholera, Staphylococcus aureus, and Bacillus cereus. *Myristica fragrans* (Nutmeg) belonging to the family *Myristicaceae*, is widely known for its medicinal properties as an appetiser and gastric stimulant. The flower extract of *Myristica fragrans* showed good antibacterial activity against *Shigella flexneri* with MIC of 26.5±0.70.The second phase of the study was aimed at exploring the potency of *Myristica fragrans* in regulating the gene expression of *Shigella flexneri*; stx-1 gene coding for the Shiga toxin wherein The PCR studies concluded the potent activity of the extract in down regulating the stx-1 gene when compared to that of the control. The significance of this study lies in the fact that there has been no research carried out so far with the flower extract of *Myristica fragrans* although potent activities have been demonstrated by the other parts of the plant

Keywords: *Foodborne pathogens, Multidrug resistance, Polymerase chain reaction, Shigella flexneri, Myristica fragrans*

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INTRODUCTION

Food poisoning due to pathogens is a major issue of public health in many developed as well as developing countries. Salmonellosis caused by the consumption of poultry, pork, and egg products infested with the bacteria Salmonella is a major concern; effective doses when ingested, the bacteria cause sickness by colonizing the intestinal tract.[1] Bacillus cereus, a toxin producing bacteria is another etiological agent responsible for 1.4%-12% of all food poisoning as well as food intoxication outbreaks worldwide. Furthermore, Staphylococcal food poisoning (SFP) is a common disease, and the number of cases had increased constantly since 1884.[2] Nutmeg (*Myristica fragrans*) belonging to the family *Myristicaceae* is an evergreen plant indigenous to Asia, Africa, the Pacific islands, and America. Traditionally the dried kernel (seed) and mace/aril are the most exploited parts of this plant and is medicinally used as a stomachic, stimulant and carminative; it is as well used for colic, headaches, diarrhea, vomiting, nausea, fever, to stimulate appetites and to control gastric problems. Nutmeg is also known to dissolve kidney stones and reduce nephritis[3] along with exhibiting analgesic, antifungal, antimicrobial, anti-inflammatory, as well as hepatoprotective activities *in vitro* and in vivo.[4] The identified and characterized compounds in Nutmeg are β- caryophyllene, safrole, myristicin, elemicin macelignan and eugenol.[5] Macelignan has been shown to possess a broad range of medicinal effects, including anti-bacterial, anti-inflammatory, and anti-cancer activity.[6]

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Most of the research studies on Nutmeg had been focused on the essential oils particularly myristicin. A very limited study has been executed on the pericarp (fruit) and aril (seed) against food borne pathogens. There has been no study so far with the flower extracts of Nutmeg so far. In view of this, the present work was executed to study the antibacterial activity of *Myristica fragrans* (aril, pericarp and flower) against Salmonella typhi, Staphylococcus aureus, and Bacillus cereus which are the common etiological agents causing food intoxications. Though the fruits, seeds and leaves of *Mystrica frangans* have been recognized for its antimicrobial activity, there has been no investigation carried out so far with the flower extracts.

MATERIALS AND METHODS

Sample Processing and Extraction

The fruits were separated into four parts i.e., fleshy pericarp (husk), aril (mace), seed-kernel (endosperm) and shell (seed-coat) (Figure 1). The dried and ground plant parts were extracted with 70% ethanol, by Soxhlet extraction.[7,8]

Figure 1: Dried flowers of *M. fragrans*

Phytochemical Screening

Qualitative and Quantitative Analysis: The presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, phlobatannins, amino acids and proteins, saponins, tannins, terpenoids, quinones, oxalates, fats and fixed oils was determined by preliminary phytochemical analysis as in Ugochukwu, 2013[9] followed by quantitative analysis as per the protocol laid by Baba et al. 2015.[10]

Antimicrobial Assay

The antimicrobial activity of the M. fragrans extract was assessed against Salmonella typhimurium (NCIM-2501), *Shigella flexneri* (NCIM-5265), Vibrio cholera (MTCC-3904), Staphylococcus aureus (ATCC-6538) and Bacillus cereus (NCIM-5557) by the well diffusion method followed by MIC determination.[11,12]

Polymerase Chain Reaction for genotypic assessment of the virulence gene

DNA sample of *Shigella flexneri* was subjected to PCR for identification of specific gene stx-1. The sample extract of *Myristica fragrans* aril was taken in the sub-MIC concentration (15.2 mg/mL). The reaction mixture was subjected to the Thermal Cycler. The following were the parameters:

- Denaturation at 95ºC for 4 min and 95ºC for 30 sec.
- Annealing of primer: 56ºC for 1 min, 72ºC for 1 min.
- Amplification was performed by 30 cycles. Primer Extension at 72ºC for 5 min. The anticipated sizes of the amplicons were determined through electrophoresis in 1.5% agarose gels, using a suitable molecular size marker, specifically a 1-kb DNA ladder; 5 µL sample with Mystrica fragrans, 5 µL sample and 3 µL of dye was run for 40 min and observed in Gel doc.
- Stx1 forward primer and reverse primer are:
- F: CAGTTAATTTGGTGGCGAAG
- R: CTGCTAATAGTTCTGCG AATC

RESULTS

Extraction of *Myristica fragrans*

Following the extraction process, total yield of the flower extract of *Myristica fragrans* was found to be 6.11 g.

Phytochemical Analysis of *Myristica fragrans*

Results obtained for qualitative analysis of phytochemicals in *Myristica fragrans* extracts are presented in Table 1 and quantitative analysis of phytochemicals are depicted in Figure 2. Of the fourteen phytochemicals screened for, the phytochemical analysis of flower extract of *Myristica fragrans* showed that they are rich in alkaloids, flavonoids and phenols.

Table 1: Qualitative Phytochemical Analysis of *Myristica fragrans* Extract

Sl. No.	Compounds	Flower extract
1	Alkaloids	+
2	Carbohydrates	+
3	Cardiac glycosides	+
4	Flavonoids	+
5	Phenols	+
6	Plobatannins	–
7	Amino acids and proteins	–
8	Saponins	–
9	Sterols	–

10	Tannins	–
11	Terpenoids	–
12	Quinones	–
13	Oxalates	–
14	Fats and fixed oils	+

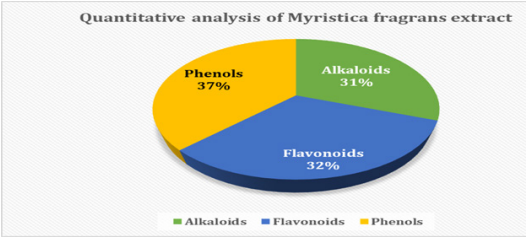


Figure 2: Quantitative analysis of phytochemicals in seed, fruit and flower extracts of *Myristica fragrans*

Antibacterial activity by well-diffusion method

The antimicrobial activity of flower extract of *Myristica fragrans* was assessed against five organisms wherein maximum activity was found against *Shigella flexneri* followed by its potent activity against S. aureus and B. cereus. The results are tabulated in Table 2.

Table 1: Qualitative Phytochemical Analysis of *Myristica fragrans* Extract

Microorganism	*Myristica fragrans* (500 mg/mL)
S. aureus	11.3±0.57
B. cereus	15±1.15
S. typhi	16±1.00
S. flexneri	24±2.00
V. cholerae	7.6±0.57

Data shown are the average and standard deviation based on triplicate runs (Mean ± Standard Deviation)

Determination of Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) of *Myristica fragrans* extract was performed against the test microorganisms at concentrations ranging from 500 mg/mL to 15.65 mg/mL. The lowest concentration which inhibited the growth of the bacterial strain was taken asthe MIC value. The MIC of *Myristica fragrans* extract against S. flexneri, S. aureus, Bacillus cereus, V. cholerae were 31.25, 31.25, 31.25 and 125 mg/mL respectively (Table 3 and Figure 3).

Table 3: Minimum Inhibitory Concentration of seed aril of *Myristica fragrans* extract.

Test organisms	Minimum Inhibitory Concentrations (mg/mL)						
	500	250	125	62.5	31.25	15.6	NC
S. aureus	11.5± 0.70	10±0	9±0	8±0	7±0	–	–
B. cereus	12.5± 0.70	9±0	8±0	–	–	–	–
S. typhi	17.5± 0.70	16.5± 0.70	15.5± 0.70	11±0	9±0	8±0	–
S. flexneri	26.5± 0.70	16.5± 0.70	14.5± 0.70	11±0	9±0	–	–
V. cholerae	17.5± 0.70	13.5± 0.70	12.5± 0.70	10±0	8±0	–	–

Data shown are the average and standard deviation based on triplicate runs (Mean ± Standard Deviation)

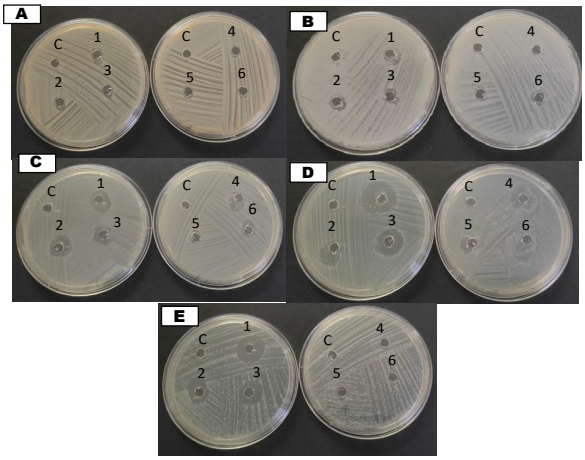


Figure 3: Minimum inhibitory concentration of of *Myristica fragrans* extract (A) *Staphylococcus aureus*, (B) *Bacillus cereus*, (C) *Salmonella typhi*, (D) *Shigella flexneri*, (E) *Vibrio cholera* 1-500 mg/mL, 2- 250 mg/mL, 3- 125 mg/mL, 4- 62.5 mg/mL, 5- 31.25 mg/mL, 6- 15.62 mg/mL

Myristica fragrans extract in regulating stx 1 gene of *Shigella flexneri*

The expression of stx-1 gene in the presence of *Myristica fragrans* extract was assessed by RT-PCR followed by Gel Doc analysis (Lane 3). A lane without the sample extract expressing the gene of interest was used for comparison (Lane 4) whereas Lane 1 consists of the DNA ladder with 100 bp which acts as the reference marker. The results showed the decreased expression of stx-1 gene in the presence of sample (Lane 3) compared to the control (without plant extract). In the presence of the extract, the stx- 1 gene bands were detected at 30 kDa with a band width of 46.3 and 53. 7 % in comparison with the control having band width of 46 and 54% respectively. The decrease in the width is the result of the potent activity of the plant extract to decrease the expression of the gene of interest in imparting virulence to *Shigella flexneri*. The depiction of bands and its qualitative analysis is shown in Figure 4 A, B and C.

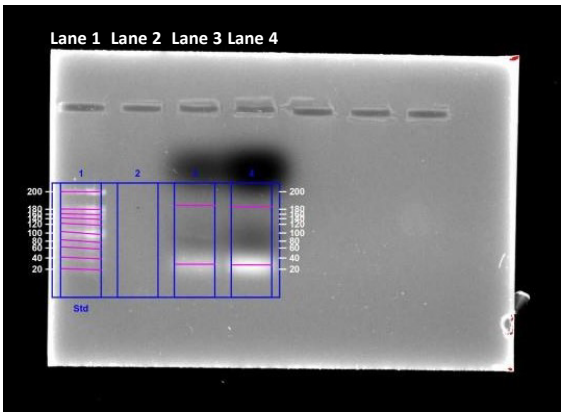
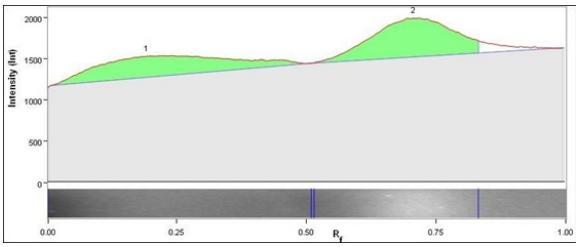


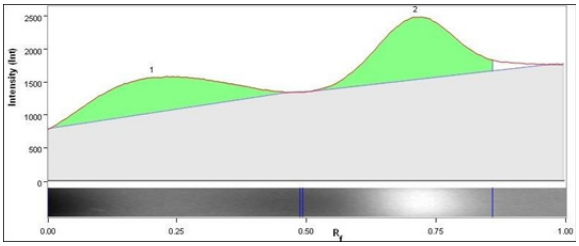
Figure 4 A: Gene Expression analysis by electrophoresis.



Lane 3

Band No.	Band Label	Base Pairs (bp)	Relative Front	Adj. Volume (Int)	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		184.1	0.197	972,010	9,644,050	N/A	N/A	46.3	44.1
2		27.2	0.710	1,127,815	7,406,490	N/A	N/A	53.7	51.2

Figure 4B: Gene expression analysis of the stx-1 gene in the presence of extract.



Lane 4

Band No.	Band Label	Base Pairs (bp)	Relative Front	Adj. Volume (Int)	Volume (Int)	Abs. Quant.	Rel. Quant.	Band %	Lane %
1		182.7	0.208	1,746,420	8,115,900	N/A	N/A	46.0	44.9
2		26.2	0.716	2,050,230	8,759,985	N/A	N/A	54.0	52.7

Figure 4C: Gene expression analysis of the stx-1 gene.

DISCUSSION

Foodborne illnesses caused by pathogens are a significant and pressing public health concern, prompting both developed and developing countries to allocate substantial resources to combat these infections. Additionally, there has been a global emergence of various antibiotic-resistant strains of S. flexneri serotypes, with a particular concern surrounding the rise of multidrug-resistant strains.

The primary objective of the study was to evaluate the antibacterial activity of *Myristica fragrans*. Additionally, the study seeks to identify a specific gene that may confer resistance or susceptibility to the antibacterial effects of these samples. The samples were subjected to Soxhlet extraction with ethanol as cited in accordance with the studies by Sulaiman et al. 2012; Rancy et al., 2015.[7,8]

Following the extraction, the extracts were subjected to antibacterial activity by Kirby Bauer method and Minimum Inhibitory concentration. The antimicrobial activity of seeds, fruits and leaves of *Myristica fragrans*[7,8] are well defined in previous studies. However, the aril flowers of *Myristica fragrans* is a new field to explore as it exhibited maximum potency against the food borne pathogens in our study.

Followed by the assessment of antibacterial activity, the effect of *Myristica fragrans* to regulate the stx- 1 gene of *Shigella flexneri* were assessed by PCR.

No prior research has been conducted on the gene regulation of food borne pathogens by *Myristica fragrans* extract.

The final outcome of the study signifies the potent property of *Myristica fragrans* in down regulating the stx-1 gene responsible for the production of Shiga toxin which is one of the virulence mechanisms of the bacteria.

Although there are studies reported with the seeds, leaves and fruit extracts of M. fragrans [4-6], there has been no prior studies or data emphasizing the antibacterial activity of *Myristica fragrans* flower extract against gastrointestinal pathogens. This study also signifies that the antibacterial actitvity of the flower extract is more potent when compared to that of the other parts of the plant. The ability of the extract in regulating the virulent gene also supports the phenotypic results of the study which was a lacunae in the previous reports and studies.

The future perspectives of this study lies in the characterization of the flower extracts and evaluating the toxicity of the compound either in vitro or in vivo followed by the formulation studies.

SUMMARY

The escalating problem of drug resistance among pathogenic bacteria has been exacerbated by the overuse of antibiotics. Researchers are exploring various innovative methods, including the utilization of naturally occurring compounds, to diminish antibacterial effectiveness and combat this challenge. Despite advancements made by the pharmaceutical industry in developing sophisticated therapeutic

strategies to treat gastrointestinal diseases effectively, there has been a significant uptick in the prevalence of enteric disorders worldwide, spanning both developing and affluent nations. This surge may be linked to the favorable environment of the gastrointestinal tract for enteric bacterial pathogens, possibly attributable to its mucous composition and the presence of macro and micro-nutrients along the epithelial cell lining.

In context to this, the antimicrobial activity of the flower extract of *Myristica fragrans* was evaluated against food borne pathogens including Salmonella typhimurium, *Shigella flexneri*, Vibrio cholera, Staphylococcus aureus, and Bacillus cereus. The extract of *Myristica fragrans* flower extract showed good antibacterial activity against *Shigella flexneri* with MIC at 31.25 mg/mL

The second phase of the study was aimed at exploring the potency of *Myristica fragrans* in regulating the gene expression of *Shigella flexneri*; stx-1 gene coding for the Shiga toxin. The PCR studies concluded the potent activity of the extract in regulating and down regulating the stx-1 gene when compared to that of the control.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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