



Determination of Antimicrobial Properties of *Myristica fragans* (Nutmeg) on Microorganisms Isolated from Veritas University Hostels Bathroom and their Surfaces

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Authors' contributions

This work was carried out in collaboration among all authors. Author TOO designed the study, wrote the protocol and the first draft of the manuscript. Author CCM managed the analyses of the study. Author EUA managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2019/v29i230157

Editor(s):

(1) Dr. Ana Cláudia Coelho, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Portugal.

Reviewers:

(1) Wafaa Abd El-Ghany Abd El-Ghany, Cairo University, Egypt.

(2) Hideharu Shintani, Chuo University, Japan.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/50164>

Original Research Article

Received 22 June 2019

Accepted 29 August 2019

Published 11 October 2019

ABSTRACT

The bathroom which is a place for people to clean up themselves is also prone to contamination. The aim of this study was to investigate the antimicrobial properties of nutmeg against isolates from the bathroom using different solvents such as acetone, ethanol and water for the extraction of the nutmeg. In this study, samples from different areas in Veritas University male and female hostel bathrooms were screened for bacterial and fungal contamination and results from the study showed that bacterial genera such as *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *Bacillus*, *Pseudomonas*, and fungus *Aspergillus* were found in this public bathroom surfaces. The Cup-plate Agar Diffusion method was used to determine the antibacterial potency of the nutmeg while the Filter paper method was used to determine its antifungal properties. The results proved that nutmeg has great anti-microbial properties thus was capable of inhibiting the growth of these isolates. Results from this study also revealed that when acetone was used as the solvent at 75% concentration, it was the most effective for the antibacterial susceptibility test while ethanoic solvent at the same concentration was the most effective for the antifungal susceptibility testing.

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Keywords: Microorganisms; *Myristica fragrans*; antimicrobial properties; agar diffusion; filter paper.

1. INTRODUCTION

Since the introduction of antibiotics, there has been tremendous increase in the resistance of diverse bacterial pathogens and this shift in susceptibility affects our ability to successfully treat patients [1].

In view of this, scientists have gone into research to find an alternative medicine. In the late 20th century, herbal medicine made a come-back as people began to seek alternatives to these drugs. Much emphasis is being given to searching for new and natural antioxidants and antimicrobials from dietary plants, because they can safeguard the human body against oxidative damage of biological macromolecules.

The use of herbals to prevent, cure and treat diseases has its existence in ancient cultures. Several plants have been reported for their many therapeutic and pharmaceutical virtues especially antioxidant, anti-tumor and anti-infectious properties [2].

Nutmeg extracts have a potential use as an antibacterial and an antifungal. Extracts from nutmeg such as its essential oils have been used as antioxidants, anti-diabetic agent, treatment of various diseases and so many others. At its antibacterial effect, it appears to single out pathogenic bacteria while leaving the other one unharmed. For example the 157 *E. coli* strain is sensitive to nutmeg extract while the non-pathogenic strains of *E. coli* are not [2].

Thus, the aim of this study is to investigate the antimicrobial properties of nutmeg against isolates (fungi and bacteria) from the Veritas University hostels bathroom using different solvents for the extraction of the nutmeg.

2. MATERIALS AND METHODS

2.1 Collection of the Plant Material

The seeds of nutmeg (*M. fragrans*) were bought from Dutse market, Abuja, cut into smaller pieces and dried for 7 days at room temperature (25°C). The dried samples were then blended into fine powder, weighed and kept away from heat, moisture and sunlight in an air-tight container.

2.2 Collection of Samples

A total of 16 samples were taken from bathrooms in Veritas University. 8 samples from the boys

hostel D and 8 from the girls hostel E bathrooms. The samples were taken from the wash hand basin, toilet seater, edge of the walls, ground centre, sewage, shower head, and door handles of the bathroom. Samples were aseptically collected using sterile swab stick. After each sample was collected, the swab sticks were inserted into peptone water for few hours to resuscitate the microorganisms. A sterile container was used to collect the water left after bathing (sewage) from the bathroom floors.

2.3 Sterilization of Materials

The materials used for this analysis were sterilized through various means depending on the nature of the material. Glass wares such as the conical flasks, beakers were sterilized using the hot air oven at a temperature of 160°C for one hour. The inoculating loops were sterilized using red hot flame, glass rods were sterilized using 70% ethanol.

2.4 Culture Media

The culture media used are; Nutrient agar Sabouraud Dextrose agar and Mueller Hinton agar. Peptone water served as a growth medium.

2.5 Isolation of Microorganisms

All media were prepared according to the manufacturer's instruction. The agars used for this experiment are Nutrient agar and Sabouraud Dextrose agar. 50 µg/ml of nystatin was added to the Nutrient agar to prevent the growth of fungi on the medium while chloramphenicol was added to the Sabouraud Dextrose agar to prevent the growth of bacteria in the medium. 0.1 ml of the inoculum was used to inoculate each of the plates labelled respectively following the spread plate method technique. The bacterial cultures were incubated at 37°C for 24 hours while the fungal cultures were incubated at 25°C by leaving on the bench for 72 hours.

2.6 Characterization of the Isolates

After incubation, the plates were observed for microbial growth and colonies were picked from the Nutrient agar plates and sub-cultured on fresh Nutrient agar plates and were incubated at 37°C for 24 hours. Their morphological identification was read and recorded. Following the observation of microorganisms, Gram's staining was performed and some biochemical

tests were done to further characterize the organisms. Already prepared nutrient agar slants were used to store the organism in the refrigerator for further analysis.

2.7 Characterization of the Fungi

The colonial morphology of the fungi was studied and observations were recorded. The fungi plates were examined by flooding lactophenol cotton blue on a slide and fungus was placed from the fungus plate by cutting part of the agar along with the fungus, teasing on the slide, covered with a cover slip and placed under the microscope. It was examined using the X40 objective lens of the microscope and the observations were recorded. The plates were kept in the refrigerator for further analysis.

2.8 Pre-extraction Preparation of Nutmeg Sample

The dried seeds of nutmeg were crushed to obtain a powdery substance using mortar and pestle and were sent to the Veritas university laboratory where it was air dried at room temperature (25°C) for about 3 days to ensure moisture removal from the sample. It was then weighed and put in an air tight container.

2.9 Preparation of Extract Concentration

Using maceration method, about 500 grams of the powdered nutmeg sample was soaked in different containers with the various solvents; ethanol, acetone and water at 3 different concentrations (75%, 50%, and 25%) in 10 ml were prepared (weight by volume).

This was done by weighing 7.5 g of the extract using the digital weighing balance (Mettler Toledo) and then dissolved in 10 ml of the solvent in a sterile glass container for 75% concentration. For 50% concentration, 5g of the extract was dissolved in 10 ml of the solvent and lastly for the 25% concentration 2.5g of the sample was dissolved in 10 ml of the solvent. This exercise was done for solvents (acetone, ethanol and water) to obtain 75%, 50%, 25% respectively. (This was done in triplicates).

The preparations were labelled appropriately and were allowed to stand at room temperature for 72 hours with frequent agitation so as to soften and break the plants cell wall to release the soluble phytochemicals. After this, the extract each was concentrated in a reduced pressure evaporator and re-constituted in DMSO [3].

2.10 Antibacterial Activities using Cup-Plate Agar Diffusion Method (Bacterial isolates)

Cup-plate agar diffusion method was adopted from (3), was modified and used. Antibacterial activity of nutmeg was evaluated using well diffusion method on Mueller-Hinton agar (MHA). The MHA plates were inoculated with the bacterial strains under aseptic conditions and agar wells were prepared with the aid of sterilized 10 mm diameter Cork borer.

3. RESULTS

The organisms on all the plates had similar characteristics.

Table 1. Morphological identification of bacterial isolates (Colonial characteristics of bacteria isolates)

| S/N | Plate code number | Shape | Elevation | Margin | Transparency | Colour | Texture | Size(mm) |
|-----|-------------------|-----------|-----------|----------|--------------|------------|---------|----------|
| 1 | TSB | Irregular | Umbonate | lobate | Opaque | Milky | Dry | 0.3 |
| 2 | DHG | Circular | Convex | Entire | Opaque | Yellow | Moist | 0.9 |
| 3 | SHG | Circular | Round | Entire | Opaque | Milky | Dry | 0.5 |
| 4 | WG | Circular | Convex | Entire | Translucent | Colourless | Moist | 0.1 |
| 5 | DHB | Circular | Convex | Entire | Opaque | Yellow | Moist | 1.1 |
| 6 | GCB | Circular | Umbonate | Entire | Translucent | White | Dry | 0.3 |
| 7 | EWG | Circular | Raised | Entire | Opaque | White | Dry | 0.1 |
| 8 | GCG | Irregular | Umbonate | Undulate | Opaque | White | Dry | 2.0 |
| 9 | SG | Circular | Round | Entire | Opaque | White | Moist | 2.3 |
| 10 | SHB | Irregular | Flat | Entire | Opaque | Greenish | Moist | 0.4 |

key: TSB= toilet seat boys hostel, DHG=door handle girls hostel, SHG=shower Head girls hostel, WG= wall girls hostel toilet, DHB=Door handle boys hostel toilet, GCB= Ground centre boys hostel toilet, EWG= Edge of the wall Girls hostel, GCG=Ground centre girls hostel toilet, Sewage girls hostel toilet, SHB= Shower head boys hostel toilet

Table 2. Morphological identification of fungal isolates (The identification of the fungi was done using 'Illustrated atlas of common plant pathogenic fungi observed microscopically)

| S/N | Plate code | Colour | Surface | Shape | Conidia surface | Size (mm) | Probable organism |
|-----|------------|--------|---------|-----------|-----------------|-----------|--------------------|
| 1 | TSB | Brown | Smooth | Glubose | Very rough | 40 | <i>Aspergillus</i> |
| 2 | DHG | Brown | Smooth | Spherical | Slightly rough | 70 | <i>Aspergillus</i> |
| 3 | SHG | Brown | Smooth | Spherical | Rough | 75 | <i>Aspergillus</i> |

key: TSB= toilet seat boys hostel, DHG=door handle girls hostel, SHG=shower Head girls hostel

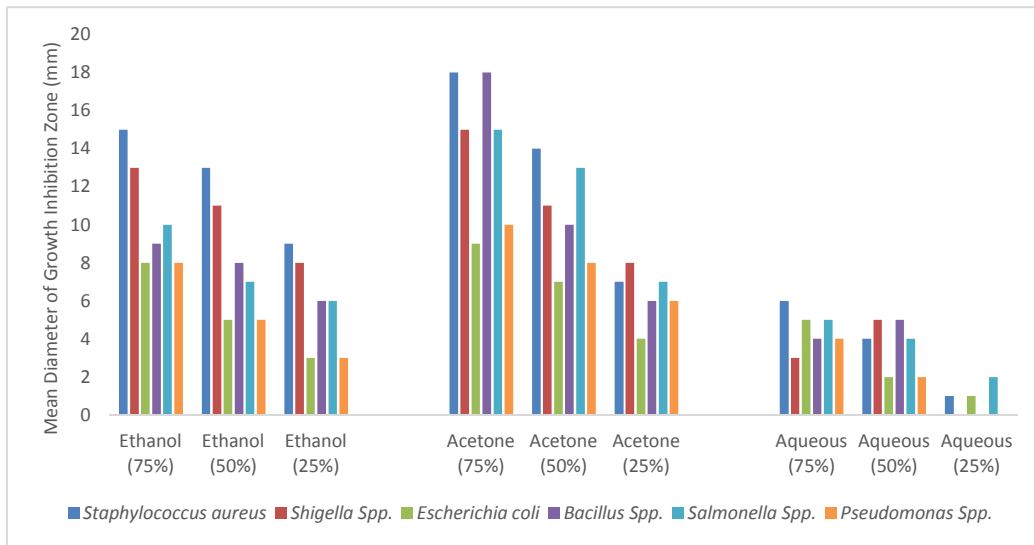


Fig. 1. Antibacterial susceptibility graph showing antibacterial susceptibility of *Myristica fragans*

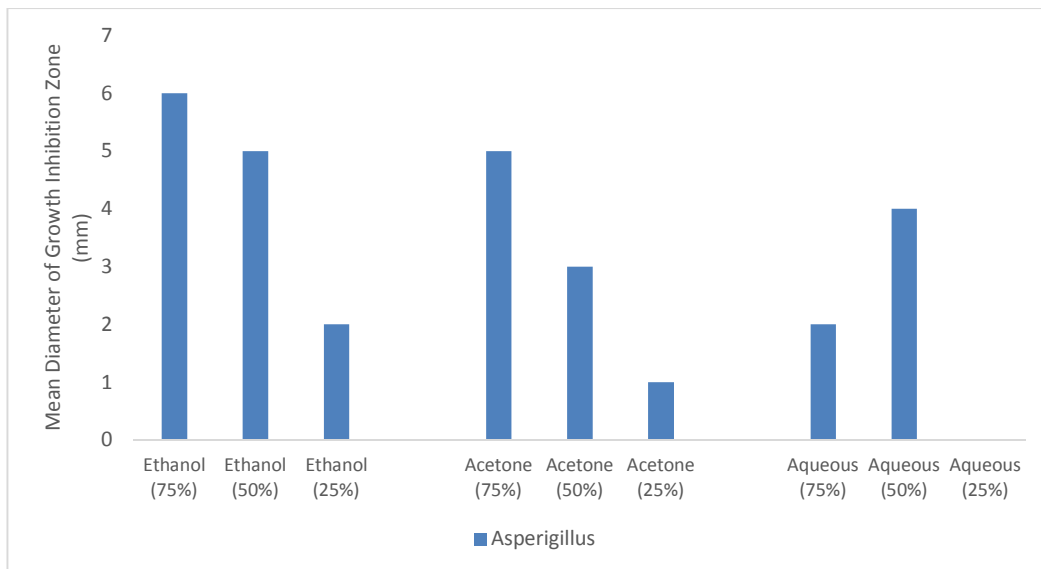


Fig. 2. Antifungal susceptibility graph showing antifungal susceptibility of *Myristica fragans*

Table 3. Gram stain and biochemical characteristics of isolates

| S/N | Isolates | Cell morphology | Grams stain | Catalase | Coagulase | Oxidase | Indole | Methyl-red | Motility | Urease | Hemolysis | Probable organism |
|-----|----------|-----------------|-------------|----------|-----------|---------|--------|------------|----------|--------|-----------|-------------------------------|
| 1 | TSB | Rod | - | + | - | - | + | + | + | - | - | <i>Escherichia coli</i> |
| 2 | DHG | Cocci | + | - | - | - | - | + | - | - | + | <i>Staphylococcus aureus</i> |
| 3 | SHG | Rod | - | + | - | - | - | + | + | - | - | <i>Salmonella enteritica</i> |
| 4 | WG | Rod | - | + | - | - | + | + | - | - | - | Shigella |
| 5 | DHB | Cocci | + | - | - | - | - | + | - | - | + | <i>Staphylococcus aureus</i> |
| 6 | GCB | Rod | - | + | - | - | + | + | + | - | - | <i>Escherichia coli</i> |
| 7 | EWG | Rod | - | + | - | - | + | + | + | - | - | <i>Escherichia coli</i> |
| 8 | GCG | Rod | + | + | - | + | - | - | + | - | - | Bacillus |
| 9 | SG | Rod | - | + | - | - | - | + | + | - | - | <i>Salmonella enteritica</i> |
| 10 | SHB | Rod | - | + | - | + | - | - | + | - | + | <i>Pseudomonas aeruginosa</i> |

key: Positive (+) = Present, Negative (-) = Absent

4. DISCUSSION

Medicinal plants have been used since ancient days to cure and prevent several diseases. This study showed that there is a possibility of contracting infections from bathrooms which are usually perceived to be a place of cleansing. The bathroom is contaminated with microorganisms from various human secretions such as saliva, skin, urine, faecal origin [4]. Positive results occurred from the door handles, toilet seat, shower head, wall, ground center, edge of the wall and sewage from the bathroom. Bacterial species such as *Escherichia coli*, *salmonella enteritica*, *Shigella*, *Staphylococcus aureus*, *Bacillus*, *Pseudomonas aeruginosa* and the fungus *Aspergillus* were isolated. This is similar to the results from the study by Ejim et al. [5] which showed the isolation of bacterial species such as *Pseudomonas aeruginosa*, *Staphylococcus auerus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Salmonella enterica*, and *Bacillus subtilis* among others from the bathroom walls of the different sampling sites with highest percentage incidence recorded for *Staphylococcus aureus*.

It was demonstrated in this study that Nutmeg (*Mystrica fragans*) has antibacterial properties and antifungal properties. It is bactericidal and fungi static. The conidia of the fungi started manifesting its growth 6 days after culturing and the growth of the colonies under test were not brightly coloured as the control and was sparsely grown. Acetone solvent was found to be the most effective compared to the other solvents. These findings are similar to that of Ashish et al. [3] where acetone extract of nutmeg was found to be significantly effective compared to other solvents because acetone extract of nutmeg seed consisted of chemicals such as sabinene, beta-pinene, mystricin, terpinen-4-ol, limonene, gamma-terpinene, p-menth-2-en-1-ol, elemicin, isoeugenol, p-menth-2-en-1-ol, myrcene, alpha-phellandrene, terpinolene, linalool and p-cymene. The compounds were extracted effectively in the solvents in the order of acetone>ethanol>aqueous. Also studies by [6] showed that nutmeg seeds exhibited strong inhibitory effects and that the effects exhibited by nutmeg may be linked to high phenolic and flavonoid contents.

5. CONCLUSION

Public bathroom is a room for diverse array of microorganisms. Surfaces such as walls, sinks,

doors, windows, flush handles and toilet seats in the bathroom are sites for microbial contamination [5]. The nutmeg is a dioecious plant, where the male and female flowers are not in one flower [7]. The seeds of nutmeg are used as culinary spice due to its flavour and preparation properties [8]. Nutmeg has been studied to having anthelmintic, hepato-protective anti-inflammatory, anti-insecticidal, anti-cancer, anti-diarrheal properties [9,2]. Studies have shown that nutmeg seeds exhibit strong inhibitory effects and that the effects exhibited by nutmeg may be linked to high phenolic and flavonoid contents. The present study showed the antibacterial and antifungal properties of nutmeg on species of *Salmonella*, *Shigella*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus* and fungal species *Aspergillus* isolated from hostel bathrooms of Veritas students'. Thus hygiene should be practiced by the public to avoid contamination by these organisms. Also more work should be carried out on the antimicrobial properties of other spices commonly found in Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Indu MN, Hatha AAM, Abirosh C, Harsha U, Vivekanandan G. Antimicrobial activity of some of the South-Indian spices against serotypes of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. Brazilian Journal of Microbiology. 2006;37:153-158.
2. Kadhim MI, Rana KN, Amaal S, Abd-Sahib . Antibacterial activity of Nutmeg (*Mystrica fragans*) seed extract against some pathogenic Bacteria. Journal of Al-Nahrain University. 2013;16(2):188-192.
3. Ashish DG, Vipin KB, Vikash B, Nishi M. Chemistry, antioxidant and antimicrobial potential of nutmeg (*Mystrica frangrans Houtt*). Journal of Genetic Engineering and Biotechnology. 2013;11(1):25-31.
4. Sherifa M. Bacterial public hazard in the public female restrooms at Taif, Kingdom of Saudi Arabia. Middle-east Journal of Scientific Research. 2013; 14(1):63-68.
5. Ejim NI, Egbuta NM, Egberongbe HO. Characterization of micro-organisms isolated from bathroom walls in a Nigerian

- University. Journal of Applied Life Sciences International. 2016;9(4):1-11.
6. Odubanjo O, Olasehinde A, Oyeleye S, Oboh G, Boligon A. Seed extracts from *Mystrica fragans* (Nutmeg) and *Moringa oleifara* (Drumstick tree) inhibits enzymes relevant to erectile dysfunction and metal-induced oxidative damage in rats' penile tissues. Journal of Food Biochemistry. 2017;42(1):35-38.
 7. Sangadji S, Kaimuddin Ala A, Paembonan SA. NUTMEG: The uniqueness of flowering and fruiting origin of Spices Islands. International Journal of Current Research in Biosciences and Plant Biology. 2015;2(3):14-18.
 8. Lopez V, Gerique J, Langa E, Berzosa C, Valero MS, Gomez-rincon C. Antihelminthic effects of nutmeg (*Mystrica fragans*) on *Anisakis simplex* L3 larvae obtained from *Micromesistius potassou*. Research in Veterinary Science 100. 2015;148-152.
 9. Pooja V, Hina S, Seema B, Ashwani KS. Targeting virulence: Novel effect of *Mystrica fragans* on Melanisation and conidiation of *Aspergillus niger*. American Journal of Drug Discovery and Development. 2012;2(1):32-39.

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Peer-review history:
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