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Research Article

Antibacterial Efficacy of Aqueous Extract of *Myristica fragrans* (Common Nutmeg)

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Abstract

Myristica fragrans (nutmeg) is a common spices used in preparation of several delicacy. This study assessed the antibacterial efficacy of *Myristica fragrans* seed against some bacteria isolates. The sample of *Myristica fragrans* was purchased from Swali market, Bayelsa state, Nigeria, and Standard Agar well diffusion sensitivity testing method was adopted for this study. The zone of inhibition for *Escherichia coli, Staphylococcus aureus, Bacillus species and Streptococcus* species was 12.7 mm, 13.3 mm, 9.3 mm and 8.3 mm, respectively for hot water extract, and 11.7 mm, 12.0 mm, 12.3 mm and 8.0 mm, respectively for cold water extract at 100% concentration. There was significant difference ($P < 0.05$) in the inhibitory properties of Myristica fragrans extracts on the various bacteria used in this study. The findings of this study showed hot water extract of *Myristica fragrans* confers superior antibacterial effect compared to cold water extracts.

Keywords: *Antimicrobial; Medicinal Plants; Myristica fragrans; Pathogens*

Introduction

Drug resistance, emerging and re-emerging microbial infections have led to search of new antibiotics source. Plants have emerged as a credible candidate for new antimicrobials. Traditionally, plants are used for the treatment of several diseases. While scientific validation have been made with regard to some of the claim of traditional medicine practitioners. It has been widely reported that about 80% of the world population rely on botanical for cure of several diseases [1-5] and most of dependant of herbs for medicine resides in rural areas in many developing countries.

According to Encyclopedia Britannica (2018), the name *nutmeg* is used to describe several seeds or fruits in different nations. For instance, *Monodora myristica* (family *Annonaceae*) is called Jamaica, calabash or nutmeg, *Cryptocarya moschata* (family *Lauraceae*) is called nutmeg in Brazil, *Laurelia aromatica* (family *Atherospermataceae*) is known as the Peruvian nutmeg, *Ravensara aromatica* (family *Lauraceae*) is known as Madagascar, or clove, nutmeg, and *Torreya californica* (family *Taxaceae*) is also called California, or stinking, nutmeg [6]. However, *Myristica fragrans has been commonly described as nutmeg* [1,7-12].

Myristica fragrans, which belongs to *Myristicaceae* family is native to Spice Islands near Indonesia, and is used as spices in several part of the world [1,8,13]. It is mainly cultivated in Penang Island, Malaysia [7], Caribbean, Southern and Western India and many other tropical nations [6,13]. Rodianawati., *et al*. [9] reported that Indonesia is the world leading producer of *Myristica fragrans. Myristica fragrans* has distinctive pungent fragrance and a warm slight sweet taste. It is used as flavour in preparation of several foods [9].

Myristica fragrans yield fruit after 7 - 9 years of planting with optimal yield being around 20 - 25 years [1] and bears fruit for about 60 years [6]. The plant can reach up to 65 feet based on height. According to Organic Facts [13], *Myristica fragrans* is a valuable plant probably due to the presence of nutmeg butter and essential oil derived from its bark and leaves, which have been acclaimed to have several health benefits.

Essential oils are secondary metabolites that are made up of volatile compounds viz: lipids, terpenoids, ketones, phenols and oxygenated derivatives [14]. Encyclopædia Britannica [6] have reported that pinene, camphene, and dipentene as the major constituents of *Myristica fragrans* essential oil (7 - 14%). Probably due to the presence of essential oil in *Myristica fragrans* it has been widely used as condiments, carminatives and in herbal medicine practice [8]. Authors have variously reported that seed of *Myristica fragrans* contain alkyl benzene derivatives (myristicin, elemicin, safrole, etc.), terpenes, alpha-pinene, beta-pinene, myristic acid, trimyristin [7,15-17], neolignan (myrislignan), and macelignan [7,18].

Myristica fragrans has been widely reported to have medicinal potentials. Traditional medicine attendants claimed that seed of *Myristica fragrans* can be used as carminative, astringent, hypolipidaemic, antithrombotic, antiplatelet aggregation, antifungal, aphrodisiac [7,19], treatment of nausea, dyspepsia [7,20], flatulence, muscle and joint pain, diarrhea, stomach ache, kidney stone, liver detoxification, boost blood circulation, digestion and respiration, appetite, and it also has anti-inflammatory properties [1]. In a review study Jaiswal., *et al*. [21] reported that chemical constituents of *Myristica fragrans* have been scientifically validated to have hypolipidemic and hypocholesterolemic activities, antimicrobial, antidepressant, aphrodisiac, memory-boosting, antioxidant, and hepatoprotective properties. Pillai., et al. [14] reported that *Myristica fragrans* also have anti-parasitic potentials.

Various studies have been carried out with regard to the antimicrobial potentials of nutmeg using ethanol, acetone, methanol, butanol, toluene, tetrahydrofuran and water as extraction solvents [1,9-12]. But information on cold and hot water extract efficacy of *Myristica fragrans* is scanty. Furthermore, most of the individuals that use it as spices added the powered form of the seed to dishes. Water is also essential in dishes prepared by addition of *Myristica fragrans*. Water is a better extraction solvent for water soluble compounds. Hence, this study aimed at assessing the antibacterial efficacy of aqueous (hot and cold water) extract of *Myristica fragrans*.

Materials and Methods

Samples procurement, preparations and extraction

Samples of *Myristica fragrans* were purchased from Swali market in Yenagoa metropolis, Bayelsa state, Nigeria. The samples were cut into pieces then macerated using pestle and mortar. 40g of the powered samples were soaked in 100 ml of water (cold and hot) for 48 hours. Then, it was filtered using muslin cloth; the filtrate was re-filtered using Whatman filter paper.

Dilution of the extracts

The extract was considered as 100% concentration and the further diluted into 95%, 90%, 85% and 80% of the original volume. This was carryout by pipetting 2.00 ml of the extract into 0.00 ml of sterile water (100%), 1.90 ml of the extract into 0.10 ml of sterile water (95%), pipetting 1.80 ml of the extract into 0.20 ml of sterile water, 1.70 ml of the extract into 0.30 ml of sterile water (85%) and 1.60 ml of the extract into 0.40 ml of sterile water (80%) [4].

Source and Preparation of organisms

The microorganisms viz: *Staphylococcus aureus, Escherichia coli, Streptococcus* and *Bacillus* species used in this study were obtained from Microbiology units, Federal Medical Centre, Yenagoa, Bayelsa state. The purity and characteristics of the bacteria isolates was checked following the scheme provided by Cheesbrough [22]. The isolates were inoculated into sterile peptone water and incubated at room temperature for 24 hours, then after it was used for the sensitivity testing.

Antimicrobial screening of the extract

The antibacterial screening of the isolates were carried out using the agar well diffusion method previously described by Lino and Deogracious [23] cited in Doherty., *et al*. [24] with slight modification by Agu and Thomas [25], Kigigha., *et al*. [4,5], Epidi., *et al* [2,3]. Nutrient agar was prepared according to the manufacturer's instruction. About 20 ml of the autoclave nutrient agar was poured on sterile Petri dish and allowed to solidify. Then 0.3 ml of the isolates was place in the solidified agar plates. Then it was spread over the surface using spreader. Sterilized cork borer was used to make three replicate holes in the agar plate with a diameter of 6 mm. 300 µl (0.3 ml) of the different concentrations of the extract was dispensed into the agar wells. The plates were labeled according. The plates were incubated for 24 hours. The resultant zones of inhibition were measured using metre rule.

Statistical analysis

SPSS software version 20 was used to for the statistical analysis. The data were expressed as Mean ± standard error. A one-way analysis of variance was carried out at P = 0.05, and Waller-Duncan Test was used to ascertain the source of the variation among the isolates.

Results and Discussion

Table 1 presents the zone of inhibition at different concentration of aqueous (cold and hot water) of *Myristica fragrans*. The zone of inhibition exhibited by *Escherichia coli, Staphylococcus aureus, Bacillus* sp. and *Streptococcus* sp. was 12.7 mm, 13.3 mm, 9.3 mm and 8.3 mm, respectively (100%), 10.7 mm, 11.3 mm, 7.7 mm and 7.7 mm, respectively (95%), 8.7 mm, 9.7 mm, 4.7 mm and 0.0 mm, respectively (90%), 8.3 mm, 7.3 mm, 0.0 mm and 0.0 mm, respectively (85%) for hot water extract, and 11.7 mm, 12.0 mm, 12.3 mm and 8.0 mm respectively (100%), 10.3 mm, 9.3 mm, 7.7 mm and 2.3 mm, respectively (95%), 7.3 mm, 7.3 mm, 0.0 mm and 0.0 mm respectively (90%), 0.0 mm, 0.0 mm, 0.0 mm and 0.0 mm, respectively (85%) for cold water extract. There was significant difference ($P < 0.05$) in most of the isolates for each of the concentrations. The cold water extract apparently have lesser effect on the test organisms. The trend reported in this study of hot water having superior effect compared to cold water extract is in consonance with the findings of Kigigha., *et al* [4]. The variation suggests that hot water have the tendency to leach out essential metabolites compared to cold water. The variation among the zone of inhibition for the various bacteria isolates tested could be associated to the cell wall characteristics of the organisms especially that of the gram negative bacteria [1], physiology, metabolism, nutrition and biochemistry of the various bacteria isolates [2,3,5].

Aqueous extract	Isolates	100%	95%	90%	85%	80%
Hot water	*Escherichia coli*	12.7 ± 0.9bc	10.7 ± 0.3bc	8.7 ± 0.9c	8.3 ± 0.3c	0.0 ± 0.0
	Staphylococcus aureus	13.3 ± 0.3c	11.3 ± 0.3c	9.7 ± 0.3c	7.3 ± 0.3b	0.0 ± 0.0
	Bacillus species	9.3 ± 0.3abc	7.7 ± 0.3b	4.7 ± 2.3b	0.0 ± 0.0a	0.0 ± 0.0
	Streptococcus species	8.3 ± 0.3ab	7.7 ± 0.3b	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0
Cold water	*Escherichia coli*	11.7 ± 0.9abc	10.3 ± 0.3bc	7.3 ± 0.3bc	8.3 ± 0.3c	0.0 ± 0.0
	Staphylococcus aureus	12.0 ± 0.0abc	9.3 ± 0.3bc	7.3 ± 0.3bc	7.3 ± 0.3b	0.0 ± 0.0
	Bacillus species	12.3 ± 3.3abc	7.7 ± 0.3b	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0
	Streptococcus species	8.0 ± 0.0a	2.3 ± 0.2a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0

Table 1: Zones of Inhibition (mm) of water extracts of Myristica fragrans.

Different letters along the column indicate significant variation ($P < 0.05$) according to Waller Duncan statistics.

The antibacterial efficacy of water extract of *Myristica fragrans* could be due to the presence of bioactive and chemical constituents [10]. Kumari., *et al*. [12], have reported that methanolic extracts of *Myristica fragrans* contain alkaloids, carbohydrates, flavonoids, terpenoids, phlobatanins and quinones and the absence of phenolic compounds, tannins and saponins [12]. Ibrahim., *et al*. [1] reported that *Myristica fragrans* 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 ingredients. Specifically, Gupta., *et al*. [11] reported that acetone extract of *Myristica fragrans* contain 32 compounds, and of these, Sabinene (28.61%), b-Pinene (10.26), a-pinene (9.72), myristicin (4.30%), isoeugenol (2.72%), p-cymene (1.81%), carvacrol (1.54%), eugenol (0.89%) and b-caryophellene (0.82%) are the contributors of its possible antioxidant and antimicrobial activity. Kumari., *et al*. [12] reported secondary metabolites that have antimicrobial potential in *Myristica fragrans.*

The antibacterial efficacy to both gram positive and gram negative organisms suggests that *Myristica fragrans* can be used for broad-spectrum antibiotics [7]. Like others solvent extracts of *Myristica fragrans* that have been variously reported to have antibacterial activity, water extract of *Myristica fragrans* also have antimicrobial effects. Nurjanah., *et al*. [26] reported that essential oil of nutmeg have antimicrobial effects against gram-positive bacteria such as *Staphylococcus aureus, Staphylococcus epidermis,* and gram-negative bacteria such as *Shigella dysenteriae, Salmonella typhi.* Nurhasanah [10] reported that methanol fruit extracts of *Myristica fragrans* is potent against *Staphylococcus aureus* and *Escherichia coli*. Gupta., *et al*. [11] reported that acetone, ethanol, methanol, butanol and water extract of *Myristica fragrans* have potent effect against some species of fungi (*Aspergillus fumigates, Aspergillus niger, Aspergillus flavus*), gram positive (*Bacillus subtilis, Staphylococcus aureus*) and negative (*Pseudomonas aeruginosa, Pseudomonas putida*) bacteria. Ibrahim., *et al*. [1] reported that ethanol and acetone seed extract of *Myristica fragrans* is potent against gram-positive bacteria viz: *Bacillus subtilis* and *Staphylococcus aureus*, while acetone, ethanol seed extracts is not potent against gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The authors further reported that water extract is not potent against both gram positive and negative bacteria. The difference in the efficacy of different study could be due to the age of the plant, environmental condition that the plant was cultivated, concentration of the extract used for the sensitivity testing, and choice of solvent [2-5, 27].

Conclusion

Plants have emerged as a credible source of new antimicrobials. This study evaluated the antibacterial efficacy of aqueous (cold and hot water) extracts of *Myristica fragrans*. The study showed that *Myristica fragrans* have antibacterial activity against both gram positive and negative organisms. As such, it could be used for broad-spectrum antibiotics. Hot water extract have apparent higher effect on the test organisms when compared to the cold water extracts.

Bibliography

1. Ibrahim KM., *et al*. "Antibacterial Activity of Nutmeg (Myristica fragrans) Seed Extracts Against Some Pathogenic Bacteria". *Journal of Al-Nahrain University* 16.2 (2013): 188-192.

2. Epidi JO., *et al*. "Antibacterial and synergistic efficacy of extracts of Alstonia boonei tissues". *British Journal of Applied Science* 1.1 (2016): 21-26.

3. Epidi JO., *et al*. "Antibacterial and synergistic potency of tissues of Vitex grandifolia". *Biotechnological Research* 2.2 (2016): 69-76.

4. Kigigha LT., *et al*. "Activities of Aframomum melegueta Seed Against Escherichia coli, Staphylococcus aureus and Bacillus species". *Point Journal of Botany and Microbiology Research* 1.2 (2015): 23-29.

5. Kigigha LT., *et al*. "Phytochemical and antibacterial activities of Musanga cecropioides tissues against Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus, Proteus and Bacillus species". *International Journal of Applied Research and Technology* 5.1 (2016): 100-107.

6. Encyclopædia Britannica. "Nutmeg" (2018).

7. Shafiei Z., "*et al*. "Antibacterial Activity of Myristica fragrans against Oral Pathogens". *Evidence-Based Complementary and Alternative Medicine* (2012).

8. Duarte RC., *et al*. "Irradiation effect on antifungal potential Myristica fragrans (nutmeg) essential oil, a preliminary study". International Nuclear Atlantic Conference-INAC (2011).

9. Rodianawati I., *et al*. "Nutmeg's (Myristica fragrans Houtt) Oleoresin: Effect of Heating to Chemical Compositions and Antifungal Properties". *Procedia Food Science* 3 (2015): 244-254.

10. Nurhasanah. "Antimicrobial Activity Of Nutmeg (Myristica fragrans Houtt) Fruit Methanol Extract Against Growth Staphylococcus aureus and Escherichia coli". *Jurnal ßIOêduKASI* 3.1 (2014): 277-286.

11. Gupta AD., *et al*. "Chemistry, antioxidant and antimicrobial potential of nutmeg (Myristica fragrans Houtt)". *Journal of Genetic Engineering and Biotechnology* 11.1 (2013): 25-31.

12. Kumari TP. "GC MS analysis and antibacterial activity of Myristica fragrans seed extracts against lower respiratory tract pathogen Acinetobacter baumanii". *Asian Journal of Pharmaceutical and Clinical Research* 7.3 (2014): 126-129.

13. Organic facts. "9 Surprising Benefits of Nutmeg" (2018).

14. Pillai S., *et al*. "Anti-parasitic Activity of Myristica fragrans Houtt. Essential Oil against Toxoplasma Gondii Parasite". *Asia-Pacific Chemical, Biological and Environmental Engineering Society Procedia* 2 (2012): 92-96.

15. Qiu Q. "Study on chemical constituents of the essential oil from Myristica fragrans Houtt. by supercritical fluid extraction and steam distillation". *Journal of Chinese Medicinal Materials* 27.11 (2004): 823-826.

16. Wang Y., *et al*. "GC-MS analysis of essential oils from seeds of Myristica fragrans in Chinese market". *Zhongguo Zhong Yao Za Zhi* 29.4 (2004): 339-342.

17. Yang XW., *et al*. "New neolignan from seed of Myristica fragrans". *Zhongguo Zhong Yao Za Zhi* 33.4 (2008): 397-402.

18. Chung JY., *et al*. "Anticariogenic activity of macelignan isolated fromMyristica fragrans (nutmeg) against Streptococcus mutans". *Phytomedicine* 13.4 (2006): 261-266.

19. Sonavane GS., *et al*. "Anxiogenic activity of Myristica fragrans seeds". *Pharmacology Biochemistry and Behavior* 71.1-2 (2002): 239-244.

20. Zaidi SFH., *et al*. "Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against Helicobacter pylori". *Journal of Ethnopharmacology* 121.2 (2009): 286-291.

21. Jaiswal P., *et al*. "Biological effects of Myristica fragrans". *Annual Review of Biomedical Sciences* 11 (2009): 21-29.

22. Cheesbrough M. "District Laboratory Practice in Tropical Countries". Low price Edition part 2. Cambridge press, England (2004).

23. Lino A and O Deogracious. "The invitro antibacterial activity of Annonna senegalensis, Sacuridecae longipendiculata and Steganotaema araliacea. Uganda medicinal plants". *Journal of African Health Science* 6.1 (2006): 31-35.

24. Doherty VF., *et al*. "Antimicrobial activities of Aframomum Melegueta (Alligator pepper)". *International Journal of Biology* 2. 2 (2010): 126-131.

25. Agu GC and BT Thomas. "Antibacterial Activities of Ethanol and Aqueous Extracts of Five Nigerian Medicinal Plants on Some Wound Pathogens". *Nature and Science* 10.2 (2012): 78-84.

26. Nurjanah S., *et al*. "Antibacterial Activity of Nutmeg Oil. in 2nd International Conference on Sustainable Agriculture and Food Security: A Comprehensive Approach". *KnE Life Sciences* (2017): 563-569.

27. Chiejina NV and JA Ukeh. "Antimicrobial Properties and Phytochemical Analysis of Methanolic Extracts of Aframomum Melegueta and Zingiber Officinale on Fungal Diseases of Tomato Fruit". *Journal of Natural Sciences* 2.6 (2012): 10-15.

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