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Studies on phytochemical analysis and antibacterial activity of *Myristica fragrans* Houtt. Shell (Fruit) extracts against selected pathogens

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Abstract

Nutmeg is a spice that has long been prized for its medicinal properties. The present study aimed to determined the phytochemical constituents and the antibacterial activity of nutmeg fruit extract against the selected pathogens. Preliminary phytochemical screening of *Myristica fragrans* Houtt. Shell revealed the existence of alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, tannins and glycosides. Results of antibacterial activity showed that ethanolic extract exhibited more antibacterial activity against the bacteria *Pseudomonas aeruginosa* (16mm). The results obtained also revealed that *Myristica fragrans* Houtt. Shell could be a valuable source of new antibacterial agent. Therefore the use of *Myristica fragrans* Houtt. fruit as spices is due to its pharmacological properties. Further research must be done to profile the various medicinal values of *Myristica fragrans* Houtt. shell and it may overlay a way to discover new drugs to cure some diseases.

Keywords: nutmeg, *Myristica fragrans* Houtt, antibacterial, phytochemical screening

Introduction

Spices are dried aromatic plant products used to flavour foods and beverages. They include leaves (rosemary, sage), flowers and flower buds (clove), bulbs (garlic, onion), rhizomes (asafoetida), fruit (pepper, cardamom), and other parts of the plant. Spices are ''Generally Recognized As Safe'' (GRAS) by the FDA, at least at concentrations commonly found in foods (Ugwuona, 2014) [13]. Spices contribute very minimal nutrients to menu because they are used a very small amount (Sunder, 2016) [11]. The bulk of the major components of spice materials consist of carbohydrate, protein and little minerals. Tannins, resins, pigments, volatile, essential and fixed oils which contribute to flavouring occur in traces and constitute only a small fraction of the dry matter (Cowan, 1999) [4].

Nutmeg is a spice that has long been prized for its medicinal properties. Nutmeg has been used for centuries in both western and eastern cultures mainly as carminative and stimulants in treating flatulence, indigestion, nausea, and other stomach as well as renal ailments. Like cloves, nutmeg contains eugenol, a compound that is believed to benefit the heart. Myristicin found in nutmeg has been shown to inhibit an enzyme in the brain that contributes to Alzheimer's disease and is used to improve memory. It has also been reportedly used in the treatment of cancers. In the Peruvian Andes, nutmeg chopped in pork fat has been used externally in massages for paralysis, rheumatism, and as an anti-parasitic. The fruit is comprised of a single seed, typically with a ruminate endosperm, covered to various degrees by a fatty white-reddish aril.

The antimicrobial activity of nutmeg oil was tested against human and plant pathogenic bacteria and fungi. The oil showed significant inhibitory activity activity against the bacteria, *Enterococcus faecalis*, *Lactococcus plantarum* and *Proteus vulgaris* and the fungus *Candida tropicalis*, *Candida albicans*, *Rhizomucor miehei* and *Candida glabrata*. No inhibitory activity was observed against the bacteria *Clostridium pertringens*, *klebsiella pneumoniae* and *Bacillus megaterium* (Helen *et al.*, 2012) [6].

Gupta *et al.* (2013) [5] were evaluated the antioxidant and antimicrobial activities of nutmeg (*Myristica fragrans* Houtt.) seed extracts. Thomas and Krishnakumari (2015) [9, 12] were evaluated the phytochemical constitution of dried seeds of *Myristica fragrans* Houtt. Using eight different solvent extracts such as methanol, ethanol, ethyl acetate, chloroform, petroleum ether, acetone and aqueous (cold and hot).

Materials and Methods

Fresh Nutmeg (fruit) of *Myristica fragrans* Houtt. Shell (Plate: 1) was collected from different locations of Surulacode village in Kanyakumari District. The plant were identified taxonomically. Human pathogenic bacteria such as *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *E. coli* were collected from Scudder Diagnostic Centre, Nagercoil. All the test bacterial species were maintained on nutrient agar media.

The fruits were washed with clean water. The shell of the fruit were removed and air dried for 5 days. The dried parts were stored in sealed and labeled containers for use. The stored shell powder of *Myristica fragrans* (10 g) was extracted with 60 ml of respective solvents namely chloroform, ethyl acetate, acetone and ethanol in sterile containers. The extract was kept in refrigerator for 4 days. Therefore, the suspensions were filtered into sterile containers separately using whatmann No.1 filter paper. The

extracts were allowed to dry at a temperature of 40 ^{0}C into powder. The powder of the extracts obtained were stored in sealed bottles and kept in a refrigerator at 4 ^{0}C until further use (Akerele *et al*., 2008) [1].

Antibacterial activity of aqueous and the solvent extracts (chloroform, ethyl acetate, acetone and ethanol) were determined by disc diffusion method on nutrient agar medium (Anonymous, 1996) [2]. Sterile Whatman filter discs (6mm diameter) were obtained from Sujha Surgicals, Nagercoil and inoculums containing bacteria were spread on the petriplates with sterile swab moistened with bacterial suspension. Then 0.1 µ1 each of all aqueous and solvent extracts were placed in the discs and placed on petriplates. The plates were incubated for 24h at 37 ^{0}C and zone of inhibition if any around the discs were measured in mm.

Preliminary phytochemical tests for the identification of alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, tannins, glycosides, carbohydrate, proteins, reducing sugar, oxalic acid, malic acid, sulphate and carbonate were carried out for all the extracts by the methods described by Khandelwal (2008) [8].

Plate 1: Selected plant for study

Result and Discussion

The antibacterial activities of *Myristica fragrans* Houtt. shell inhibited the growth of selected bacterial pathogens such as *Bacillus subtilis*, *Enterococcus faecalis* and *Staphylococcus aureus* were gram positive pathogens and *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli* were gram negative pathogens for the study. The different solvent selected for the study were acetone, benzene, diethyl ether, ethanol, ethyl acetate and aqueous. Amikacin was the control used in our study.

The phytochemical analysis of *Myristica fragrans* Houtt. shell is shown in (Table: 1; Fig: 1). The maximum number of compounds (7) present in the extract of ethanol. The ethanol extract revealed the presence of phytochemicals such as alkaloids, flavonoids, phenols, saponins, steroids, tannins and glycosides. The less number of phytochemicals (5) were present in ethyl acetate and benzene extracts. Preliminary phytochemical analysis helped to identify therapeutic compounds in plants. In this study, secondary metabolites such as saponin, alkaloids, tannins, phenol, flavonoids and steroids were present in different extracts of *Myristica fragrans* Houtt. Shell which is in agreement with the studies carried out by Assa *et al.*, (2014) [3]; Rancy and Krishnakumari (2015) [9, 12]. Ethanol is an excellent organic solvent for extraction of metabolites from *Myristica fragrans* Houtt. Fruit seed due to the fact that ethanolic extract contained more phytoconstituents than ethylacetate and aqueous extracts respectively (Jimoh *et al.*, 2017) [7].

The antibacterial activity of *Myristica fragrans Houtt.* Shell exhibited the maximum inhibition zone of (16mm) recorded in ethanol extract against *Pseudomonas aeruginosa*. The ethanol extract showed significant activity of 15mm against *Staphylococcus aureus*. The ethanol extract showed inhibitory activity of 14mm against *Enterococcus faecalis* and 13mm against *Enteroccus faecalis* in ethyl acetate extract and *Staphyloccus aureus* in acetone extract. Minimum inhibitory activity was recorded in acetone extract (Table: 2; Fig. 2; Plate: 1). Earlier report also showed high antimicrobial activity of *Myristica fragrans* Houtt. Flesh, mace and seed extracts against some pathogens (Shafiei *et al.* 2012) [10].

Drugs to cure some diseases.

Table 1: Phytochemicals constituents in shell portion of*Myristica fragrans* Houtt. fruit

Phytochemicals	Solvent extracts					
	Ethanol	Ethyl acetate	Acetone	Benzene	Diethyl ether	Aqueous
Alkaloids	+	+	++	+	+	+
Flavonoids	+++	++	+++	-	+	++
Phenols	+++	+++	+++	+	++	+++
Saponins	+	+	+	+	+	+
Steroids	+++	++	++	+	-	++
Terpenoids	-	-	-	-	+	-
Tannins	+	-	+	-	+	++
Glycosides	+	-	-	+	-	-
Reducing sugars	-	-	-	-	-	-
Proteins	-	-	-	-	-	-

(+++) - High (++) - Medium (+) - Low (-) - Absent

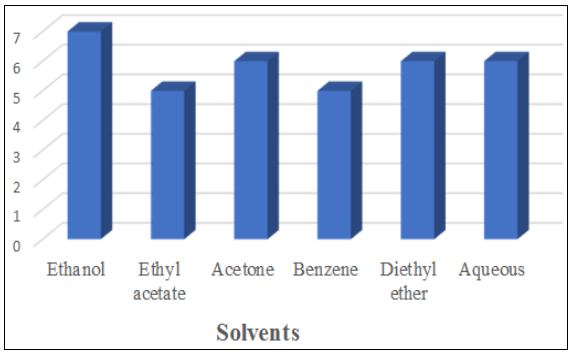

Fig 2: Phytochemical analysis of *Myristica fragrans* Houtt. Fruit shell

Table 2: Shell portion extract of *Myristica fragrans* Houtt. fruit showing antibacterial activity

Pathogens	Zone of inhibition (mm) in different solvent						
	Ethanol	Ethyl acetate	Acetone	Benzene	Diethyl ether	Aqueous	Control
Bacillus subtilis	10mm	11mm	10mm	-	10mm	-	20mm
Escherichia coli	10mm	-	7mm	10mm	-	-	21mm
Enterococcus faecalis	14mm	13mm	7mm	11mm	-	12mm	20mm
Pseudomonas aeruginosa	16mm	12mm	9mm	7mm	9mm	9mm	24mm
Salmonella typhi	12mm	8mm	7mm	8mm	12mm	-	29mm
Staphylococcus aureus	15mm	9mm	13mm	10mm	12mm	-	20mm

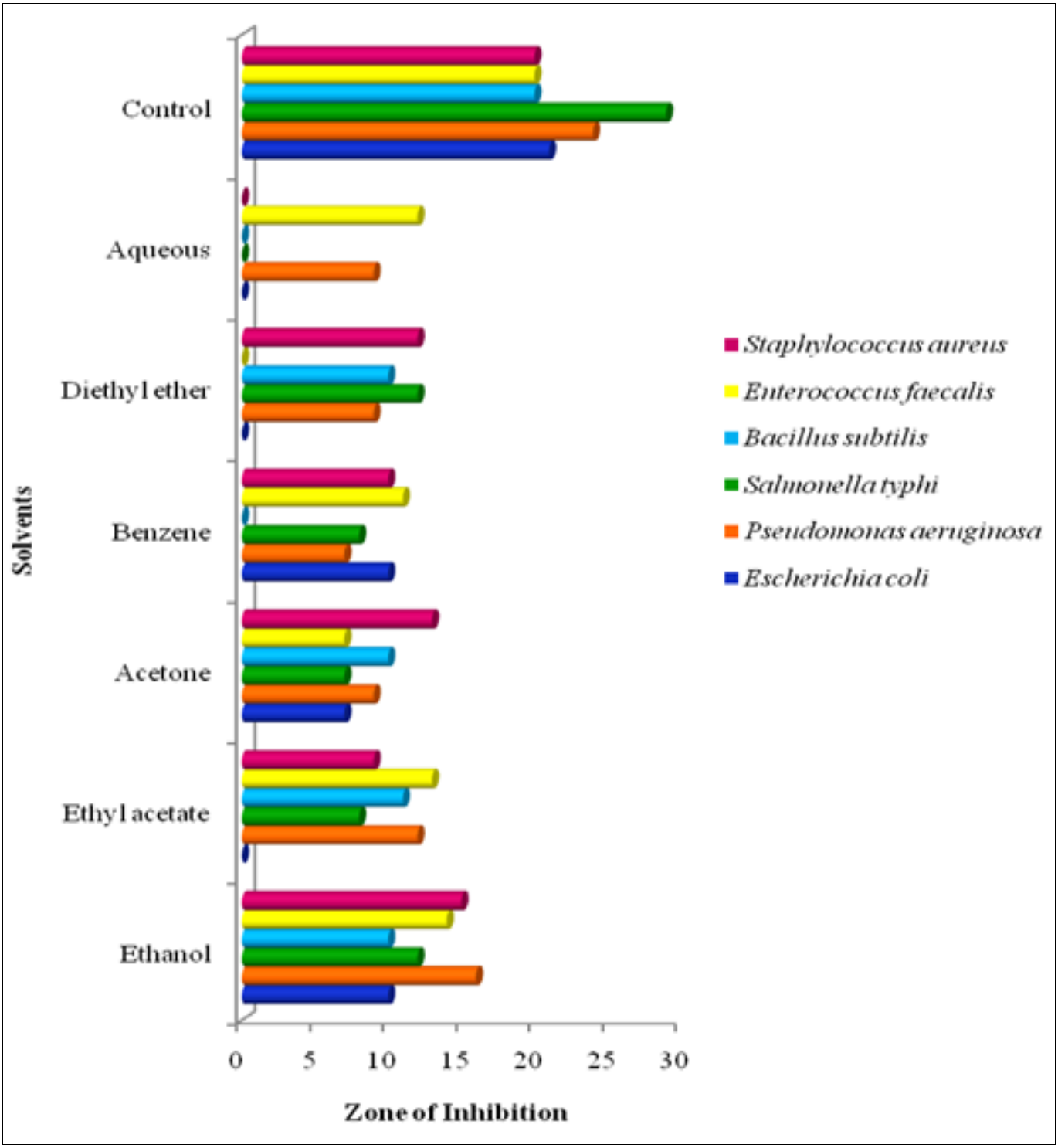

Fig 3: Shell extract of *Myristica fragrans* Houtt. fruit showing antibacterial activity against various tested pathogens

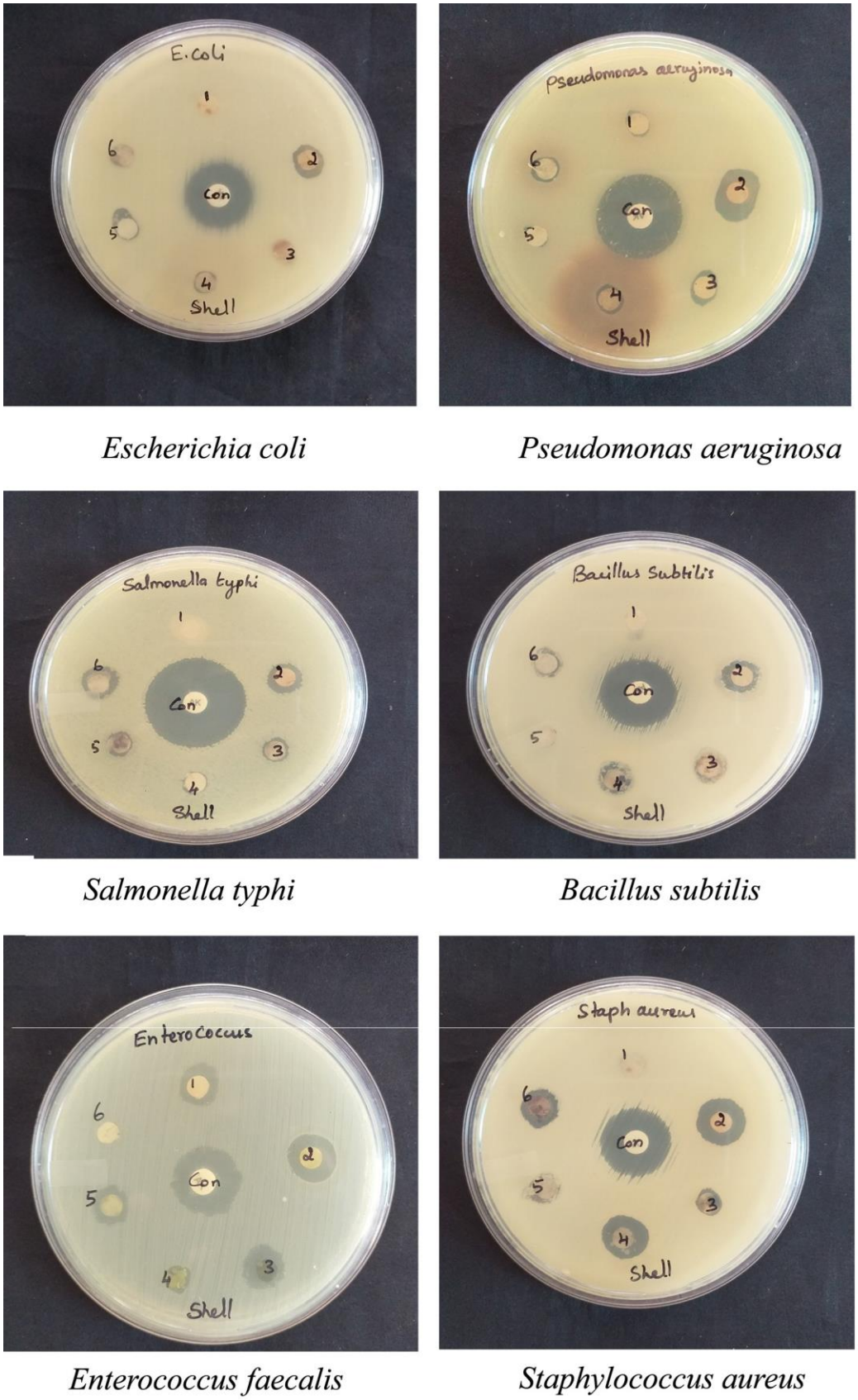

Plate 2: Shell extract of *Myristica fragrans* Houtt. Fruit showing inhibitory activity against the selected test organism

Summary and Conclusion

The present work showed that the ethanol extract of *Myristica fragrans* Houtt. shell showed highest activity than any other extracts and *Pseudomonas aeruginosa* was highly inhibited, The least activity was noted in the acetone, diethyl ether and aqueous extracts. In all the tested extracts aqueous extracts showed least activity. And some of the extracts showed no activity against tested pathogens. The results obtained also revealed that *Myristica fragrans* Houtt. could be a valuable source of new antibacterial agent. Therefore the use of *Myristica fragrans* Houtt. as spices is due to its pharmacological properties. Further research must be done

to profile the various medicinal values of *Myristica fragrans* Houtt. fruit and it may overlay a way to discover new drugs to cure some diseases.

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