

## NUTMEG: A PROMISING ANTIBACTERIAL AGENT FOR STABILITY OF SWEETS

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### ABSTRACT

The growing concern about safety of foods has recently led to the development of natural antimicrobials to control the food-borne pathogens. Spices are one of the most commonly used antimicrobial agents in foods not only imparts flavor and pungent stimuli but also provides antimicrobial property. The objective of the study is to evaluate antimicrobial activity of methanolic extract of *Myristica fragrance* Houtt. and *Acorus calamus* Linn. To know the Phytochemical present in the methanolic extract of both the drugs so as to correlate the components responsible for the said activity and also to evaluate the herbal drugs by morphological characteristics. The minimum inhibitory concentration of nutmeg and acorus extracts were found to be; 300 and 250 µg/ml. It is interesting to note that the both the extract were found to contain almost similar phytochemical. Hence the study supports the addition of nutmeg powder in sweets like *ladoo*, *kheer* etc.

**Key words:** *Myristica fragrance*, *Acorus calamus*, Antimicrobial activity, phytochemical evaluation.

### 1. INTRODUCTION

Present study is focused on two crude drugs viz., dried seed kernels of *Myristica fragrance* Houtt. family-Myristaceae (*Jaiphal*) and rhizomes of *Acorus calamus* Linn, family-Araceae (*Vekhand*)<sup>1-4</sup> as nutmeg is used traditionally in preparation of sweets whereas vekhand is used as anti-inflammatory. *Myristica fragrance* (Figure 1) is used as a spice and has anti-inflammatory properties<sup>5</sup> and can be used to treat joint and muscle pain. It act as an excellent liver tonic.

Nutmeg oil is also a good herb for the kidney, helping it dissolve kidney stones as well as relieve infections of the kidney, also act as anti-diarrhoeal<sup>6</sup>. Nutmeg tree is slow-growing evergreen grows to more than 20 m and is cultivated in India, Ceylon, Malaysia and

Granada. The fruit, which is called a drupe or a nutmeg apple, is similar in appearance to a peach or an apricot. When the mature fruit splits open, the nutmeg (stony endocarp or seed surrounded by a red, slightly fleshy network or aril) is exposed. The dried aril alone is called mace. The nut is removed and dried to produce nutmeg.

*Acorus calamus* (Figure 2) has long been used as a digestant, an expectorant, stimulant against digestive disorders, and treatment of diarrhea, also used as an insecticidal agent<sup>7</sup>. *Acorus calamus* and the essential oil of its rhizome serve mainly as an insecticide and insect repellent. Indeed, the rhizomes of Indian *Acorus calamus* possess insecticidal, larvicidal, antitermite and larvae repellent and

insect-repellent properties. In Ayurvedic medicine, the rhizomes are considered to possess antispasmodic, carminative and anthelmintic properties and are used to cure many disorders such as epilepsy<sup>8</sup>.

Scientific experiments on the antimicrobial properties of some plants and their components have been documented in the late 19<sup>th</sup> century so there is a need to explore this aspect of drugs we have selected; nutmeg and acorus. However, several plants are used in India in the form of crude extracts; infusions are plaster to treat common infection without scientific evidence of efficacy.

In present study we are focused on natural antimicrobial agents and evaluating the crude drugs by morphology and by phytochemical means. Also to establish the relationship between phytochemical in the extracts to the antimicrobial activity.

The current era of anti-microbial therapy began more than 60 years ago. The use of anti-microbial agents, antibiotics and chemical drugs has greatly contributed to the improvements in health. However, the indiscriminate use of anti-microbial agents has posed a serious problem of "anti-microbial resistance". Antimicrobial resistance is a global problem that needs urgent action. Infectious agents are the world's leading killers after cardio-vascular diseases as they account for 13.3 billion deaths, which is approximately 25% of the total global deaths (World Health Organization). Many plants used today were well known to the people of ancient cultures throughout the world for their preservative and medicinal powers.

## 2. MATERIALS AND METHODS

### 2.1.1 Plant material

The dried seed kernel of *Myrestica fragrance* Houtt. and rhizomes of *Acorus calamus* Linn. were purchased from local market of Santacruz, Mumbai-400 098 and send for authentication at Agarkar Research Laboratory, Pune. The authentication report no. were; Autn. No. 08-124 and 08-125 for *Acorus calamus* and *Myristica fragrance* respectively. The voucher specimen of both drugs are deposited in the museum of Bombay college of Pharmacy, Kalina, Mumbai-98.

### 2.1.2 Chemicals

Methanol, dimethyl sulfoxide, petri-plate, test tubes, micropipette, inoculating loop. All other chemicals used were of analytical reagent (AR)

grade; freshly prepared distilled water was used wherever needed.

### 2.1.3 Microorganisms

Strains of Microbes used are ; Gram positive- *Staphylococcus aureus*, *Beta coagulans*, *Beta subtilis*, *Beta cereus* ; Gram negative- *Escherichia coli.*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*; Fungi- *Candida albicans*, *Aspergillus niger*, *Saccharomyces cerevisiae*.

### 2.2 Extraction

The course powder material of seed kernel of Nutmeg was subjected to Soxhlet extraction using methanol as a solvent (1: 9), at optimum temperature and recycled (six cycles) until extraction was completed, then plant extracts were filtered and concentrated to dryness on steam bath at optimum temperature to get the nutmeg methanolic extract (NME)<sup>9, 10</sup>. Same procedure was followed for Acorus using methanol as a solvents to get the Acorus methanolic extract (ACME).

### 2.3 Morphological and Phytochemical Evaluation

Both the crude drugs were evaluated by colour, odor, taste, size, shape and special features like touch, texture and compared with the standards given in official books (Table-1). The NME and ACME were subjected to chemical tests as per the official tests for the identification of the various phytochemical<sup>11</sup> and the results are documented in the form of Table 1 and Table 2. Further presence of myristicine and asarones in NME and ACME was confirmed by following confirmatory tests;

Powdered nutmeg on micro sublimation produces sublimes of colourless crystals of myristicin.

Presence of asarones in Acorus is confirmed by observing blue fluorescence when the spot on TLC is exposed to UV light at 254nm.

### 2.4 Antimicrobial activity

Procedure-The NME and ACME stock solutions 1mg/ml were prepared using dimethyl sulfoxide. The dilutions for MIC (Minimum Inhibitory Concentration) determination were serially done upto a concentration of 1000 µg/ml. Similarly standard drug solution was prepared using amoxicillin in distilled water to get final concentration 400 µg/ml. MIC for antibacterial activity was determined by spot inoculation

method and antifungal activity was determined by agar slant method<sup>12, 13</sup>.



Fig. 1: Seed kernel of Nutmeg



Fig. 2: Acorus plant with rhizomes

### 3. RESULT AND DISCUSSION

The given drug samples were authenticated as *Acorus calamus* Linn. *Myrestica fragrance* Houtt., and were evaluated by morphological characters (Table 1) and their extracts were evaluated by phytochemical analysis (Table 2). The MIC Values of NME and ACME were found to be 300 µg/ml and 200 µg/ml, which indicates that the low concentration of drug is required for antimicrobial activity.

From above data, it is found that tannins, phenol and terpenoids present in ACME are showing antimicrobial activity against *S. aureus*, *B. cereus* and antifungal activity against *C. albicans*, *S. cervisia* whereas tannins, phenol and terpenoids present in NME are showing antimicrobial activity against *S. aureus*, *E. coli*, *K. pneumonia*. ACME has shown antifungal activity but NME failed to show any activity against fungi.

Table 1: Evaluation of *Myristica fragrance* and *Acorus calamus*

Evaluation	Properties observed	
	<i>Myrestica fragrance</i> seed kernel	<i>Acorus calamus</i> rhizome
Morphological evaluation	Color- Faint yellow to colorless Odor- Characteristic, nauseating Size-2-3 cm long and 2 cm wide Shape -Broadly oval Extra features- Special character "chalaza" which is a small circular depression at one end of seed was observed.	Color-Pale to dark brown Odor-Aromatic, sweet Size-7cm -12 cm long and 1.2 -1.8 cm in diameter Shape- Cylindrical. Surface-Wrinkles, circular root scars were observed. Extra features-Small, raised circular root scars were present.
Phytochemical evaluation of NME and ACME	Carbohydrates (Reducing sugars, hexose sugars, pentose sugar, starch, gums), tannins containing glycosides, phenols, proteins, flavenoids, fats (lipids), volatile oil containing terpenoids and fixed oil, tyrosine (amino acid), saponins.	Carbohydrates (Reducing sugars, hexose sugars, pentose sugar, starch, gums), mucilage, phenols, flavenoids, fats (lipids), volatile oil containing terpenoids and fixed oil, saponins.

**Table 2: Phytochemical investigation of NME and ACME**

S. No.	Test for Phytochemical	Observation for NME	Inf. NME	Observation for ACME	Inf. ACME
1	Carbohydrates-				
a)	Molish test- Extract + alpha naphthol+ add conc. Sulphuric acid along the sides of the test tube.	Reddish violet color obtained at the junction between two liquids.	√	Violet color seen at the junction	√
b)	Fehling's Test-To the extract solution ,equal quantity of Fehling's solution A and Fehling's solution B were added, then heat gently	Brick red precipitate obtained	√	Brick red precipitate obtained	√
2	Glycosides				
c)	Legal test- Extract was dissolved in pyridine, and then sodium nitroprusside solution was added to it and made alkaline.	Pinkish red color was produced.	√	---	x
d)	Baljit test- To the drug extract, sodium picrate solution was added	Yellow to orange color was produced	√	---	x
e)	Borntrager test-To the test solution added a few ml of dilute sulfuric acid. Boiled the filtrate and extracted the filtrate with ether or chloroform. Then organic layer was separated to which ammonia was added.	Pink color was observed in organic layer.	√	---	x
3	Saponins				
f)	About 1 ml of alcoholic extract was diluted separately with distilled water up to 20 ml and shaken in graduated cylinder for 15 minutes.	1 cm layer of foam was observed.	√	1 cm layer of foam was observed.	√
4	Tannins				
g)	To the sample of the extract, ferric chloride solution was added	Greenish black color was produced	√	---	x
h)	To the sample of the extract, potassium cyanide solution was added	Deep red color was produced	√	--	x
5	Proteins and amino acids				
i)	Biuret test- Added 1 ml of 40 % sodium hydroxide and 2 drops of 1 % copper Sulphate to the extract	Violet color was produced	√	---	x
j)	Ninhydrine test- Added 2 drops of freshly prepared 0.2 % ninhydrine reagent to the extract and heated.	Blue color was developed	√	---	x
6	Oil				
k)	Transverse section+ Sudan red -III solution, allow to stand for 10- 15 minutes.	The oil globules were stained pink	√	The oil globules were stained pink	√

√- indicates phytochemical present; x- indicates phytochemical absent.

## 5. CONCLUSION

The present study reveals that the ACME and NME shows significant protection against the disease causing microbes. When we compared the phytochemical data, it is surprising to know that both extracts contains similar phytochemical for the said activity. The study also shows that the *Acorus calamus* is better antifungal agent than *Myrestica fragrans*. This study supports that the addition of these herbs in food helps for antimicrobial activity and results in preservation of food for long time which can be illustrated as, "addition of potent

nutmeg powder in sweets like *ladoo*, *payasam*, *kheer* not only imparts flavour but also stabilize the preparation for long days by imparting antimicrobial property".

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