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STUDY OF INHIBITION OF FOOD CONTAMINANT BACTERIA BY Myristica fragrans Houtt. EXTRACTS

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Abstract: Myristica fragrans Houtt. commonly known as Nutmeg (Family: Myristicaceae) is a widely used food spice and flavouring agent. The nutmeg tree is important for two spices derived from the fruit: nutmeg and mace. Food items get contaminated very easily by microorganisms. Myristica fragrans Houtt is known to exhibit some antibacterial properties. The present study aims to evaluate the anti-bacterial activity of extracts of Myristica fragrans Houtt on common food contaminating bacteria like Bacillus cereus and Staphylococcus aureus. Cold extracts of Nutmeg were prepared by using 70%Ethanol and 70% Chloroform as solvents on rotary shaker for five days. All the extracts were then evaporated on flash evaporator and residue thus obtained was reconstituted in DMSO (Dimethyl sulfoxide). The extracts thus obtained were considered to be 100% extract for both ethanol residue and chloroform residue. These extracts were converted into three dilutions namely 1%, 10% and 100% using DMSO as vehicle. The above mentioned dilutions were utilized to assess antimicrobial activity by agar well diffusion method. It was observed that 10% Chloroform and 10% Ethanol extract inhibited growth of Bacillus cereus and Staphylococcus aureus. Comparison of the inhibition pattern for chloroform and ethanol extracts exhibited greater zone of inhibition as the concentration increased from 1% to 100%. However, 10%Chloroform extract was found to be more effective against Staphylococcus aureus and Bacillus cereus than 10%Ethanol extract. The MIC studies revealed that ethanolic extract was more potent in inhibition of Bacillus cereus and Staphylococcus aureus.

Index Terms - Myristica fragrans Houtt, 10% Chloroform extract, 10% Ethanol extract, Staphylococcus aureus, Bacillus cereus, food contaminants, MIC

I.INTRODUCTION

Many plant species have been commonly used as spices and flavouring agents in Indian households for generations. Since many of these plants have been used for centuries as a regular constituents of the diet in lesser quantities, they are assumed to be without side effects. The antimicrobial activity of some regularly used spices and medicinal plants such as Coriandrum sativum, Eugenia caryophyllus, Zingiber officinale, Mentha piperit, Thymus capitatus, Rheum officinale. Curcuma domestica and Cinnamomum zeylanicum [1,2] have been studied on specific species of microbes. But their specific mode of action is not studied. This antimicrobial activity of the spices can be utilized to find a solution against food contaminant bacteria which are otherwise tackled with the help of harmful chemicals.

Myristica fragrans Houtt. commonly known as Nutmeg (Family: Myristicaceae) is a widely used food spice and flavouring agent that has received attention as an alternative hallucinogen. Nutmeg has been used in Indian cooking and folk medicine. The folk uses of nutmeg have included the treatment of gastric disorders and rheumatism, and it has been used as a hypnotic and an aphrodisiac [3]. During the 6th century AD, nutmeg was imported by Arab traders. By the 12th century, these spices were well known in Europe. At the turn of the 19th century, interest developed in the use of nutmeg as an abortifacient and a stimulant for menses. These properties have been largely discounted but remain a persistent cause of nutmeg intoxication in women [4]. Many medicinal properties of Nutmeg like effects on CNS [5], antimicrobial activities [6], antioxidant activity [7] and anti-cancer properties [8] etc. have been studied.

The World Health Organization data shows that about 2.2 million people become ill on daily basis around the globe due to more than 200 foodborne diseases and about two-thirds of the outbreaks that occur originate in their homes and in catering establishments [9].The per capita food loss in Europe and North-America is 280-300 kg/year. In Sub-Saharan Africa and South/Southeast Asia it is 120-170 kg/year. The total per capita production of edible parts of food for human consumption is, in Europe and North-America, about 900 kg/year and, in

sub-Saharan Africa and South/Southeast Asia, 460 kg/year [10]. One of the major factors of wastage of cooked food is the growth of microorganisms. According to the Iyekhoetin Matthew Omoruyi et al., 2012[11] foodborne diseases have been shown to have direct impact on the health and welfare of a large number of the world population. The in vitro antibiogramic properties of natural spices on common food borne pathogen became necessary both in improving food safety and development of new drugs [12]. In the present study an effort has been made to study the effect of two different types of extracts of Myristica fragrans on bacterial species like Staphylococcus aureus and Bacillus cereus.

II. RESEARCH METHODOLOGY

2.1 Collection of plant material and preparation of extracts

Nutmeg seeds were purchased from local market and dried at room temperature for five days. A powder was made with mortar and pestle. This powder was further used for all the experimental purpose. Two commonly food contaminant microbes were selected for performing antimicrobial activity. Pure cultures of Bacillus cereus (NCIM NO. 2155) and Staphylococcus aureus (NCIM NO. 2079) were produced from National Collection of Industrial Microorganism (NCIM), Pune. These cultures were maintained on sterile Nutrient Agar Slants.
Myristica fragrans Houtt is known to contain volatile essential oils which has a high risk of evaporation during Soxhlet extraction therefore rotary shaker was used for the extraction procedure set at 370C. 70% Ethanol and 70%Chloroform were used as a solvent for extraction, 10 gm of Nutmeg Powder was weighed and it was placed in two separate flasks containing 250 ml of solvents respectively. Each flask was then kept in shaker at 100 rpm at 370C for 5 days. With each day 50 ml of fresh solvent was added, and previous extract was collected and then filtered through Whatman filter paper. This filtrate was then transferred to the pre-weighed glass beaker and stored in fridge. After 5 days of extraction, the extracts were evaporated on flash evaporator to obtain residue. The weight of the residue was recorded. The residue was reconstituted in DMSO (Dimethyl sulfoxide) which is a colourless liquid. It is an important polar aprotic solvent that dissolves both polar and nonpolar compounds and is miscible in a wide range of organic solvents as well as water [13]. The extracts thus obtained were considered to be 100% extract for both ethanol residue and chloroform residue. For assessment of antimicrobial activity these extracts were used by agar well diffusion method.

2.2 Study of Antimicrobial Activity of Myristica fragrans Extracts

Antimicrobial activity of ethanolic and chloroform seed extracts of Myristica fragrans Houtt was evaluated by Agar well Diffusion method. This study helped in ascertaining the zone of inhibition produced by 70% Ethanol and 70%Chloroform extracts of Myristica fragrans Houtt and dilutions of these extracts. Sterile nutrient agar plates were prepared and wells were made in these plates by sterile gel borer. In each of these wells 1ml of 1%, 10% and 100% Ethanolic extract reconstituted in DMSO was added aseptically along with the control (DMSO). Same procedure was performed for chloroform extracts. All the experimental sets were prepared in triplicates. These plates were incubated at 370C maintaining sterile condition for 24 hrs and then checked for zone of inhibition.

2.3 Determination of Minimal Inhibition Concentration (MIC) of Myristica fragrans Extracts

In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (like an antifungal, antibiotic or bacteriostatic) drug that will inhibit the visible growth of a microorganism after overnight incubation. MICs can be determined on plates of solid growth medium like agar or broth dilution methods in liquid growth media after a pure culture is isolated. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Because a lower MIC value indicates that less of the drug is required in order to inhibit growth of the organism, drugs with lower MIC scores are more effective antimicrobial agents [14].

In the present study, for determination of MIC of Myristica fragrans Houtt extracts, broth dilution methods in liquid growth media was utilized. Nutmeg seed powder was extracted with 70% Ethanol and 70% Chloroform as mentioned above. The extracts thus obtained were evaporated on flash evaporator and were reconstituted in 25% Nutrient Broth for MIC. This extract was serially diluted in the preinoculated cultures of Bacillus cereus and Staphylococcus aureus from 0.1 ml to 1ml. All the tubes were incubated in the incubator at 370C for 24 hrs. After 24 hours, 0.5 ml of 0.1% TTC solution (2, 3, 5-Triphenyl tetrazolium chloride) was added in all tubes and incubated for 30 min at room temperature. Microbial growths were determined by observing the change in colour in the test tubes. The microbial growth is indicated by formation of pinkish-red formazon whereas no colouration indicates inhibition of microorganisms by extracts.

III. RESULTS AND DISCUSSION

Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganisms especially the food contaminant bacteria. These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [1,2].

To study antimicrobial activity of Myristica fragrans, two types of extracts were utilized namely 70% Ethanol and 70%Chloroform. They were evaporated and residue thus obtained was reconstituted in DMSO and used for preparation of doses for the screening of antimicrobial activity against two common food contaminant bacteria Bacillus cereus and Staphylococcus aureus.

The results for the study are summarized in the following table.

Table 1: Results of Antimicrobial Activity of Myristica fragrans Extracts

Serial No	Microorganism used for screening	Zone of inhibition (Average) in cm for different concentration of Chloroform Extracts in DMSO			Zone of inhibition (Average) in cm for different concentration of Ethanol Extracts in DMSO		
		1%	10%	100%	1%	10%	100%
1.	Bacillus cereus	1.2	1.3	1.2	1.7	1.8	1.7
2.	Staphylococcus aureus	1.7	1.8	1.7	1.7	1.6	1.7

The results from the table reveal that 1%, 10% and 100% chloroform extract of Myristica fragrans reconstituted in DMSO shows greater antimicrobial activity against Staphylococcus aureus than Bacillus cereus. Whereas, it was observed that 1%, 10% and 100% ethanol extract of Myristica fragrans reconstituted in DMSO exhibited comparable antimicrobial activity against both Bacillus cereus and Staphylococcus aureus.

The above mentioned 70% Ethanol and 70%Chloroform extracts of Myristica fragrans seed were used for the estimation of MIC against Bacillus cereus and Staphylococcus aureus.

The results for this study are tabulated as follows

Table 2: Minimum inhibition concentration of Chloroform and Ethanol extract

Organism	%Concentration of Chloroform extract									
	12.5	6.25	3.125	1.562	0.781	0.390	0.195	0.097	0.048	0.024
Bacillus cereus	_	+	+	+	+	+	+	+	+	+
Staphylococcus aureus	_	_	+	+	+	+	+	+	+	+
Organism	%Concentration of Ethanol extract									
	12.5	6.25	3.125	1.562	0.781	0.390	0.195	0.097	0.048	0.024
Bacillus cereus	_	_	_	+	+	+	+	+	+	+
Staphylococcus aureus	_	_	_	_	_	_	_	_	+	+

+: Growth (no inhibition)
_: No Growth (inhibition)

The results show that both chloroform and ethanol extracts of Myristica fragrans exhibit inhibition of Bacillus cereus and Staphylococcus aureus. It was also observed that Ethanol extract showed comparatively greater MIC against Bacillus cereus and Staphylococcus aureus than chloroform exrtract. Also it is worth noticing that even at lower of concentration 0.097% the ethanolic extract inhibit the growth of Staphylococcus aureus.

The observations of the present study thus confirms the use of spice like Myristica fragrans Houtt against common food contaminants like Bacillus cereus and Staphylococcus aureus. It not only adds flavor and fragrance to the food items but also increases their shelf life without the side effects of synthetic food preservatives. Thus, it can be concluded that Myristica fragrans Houtt can be developed and used as alternative food preservative. It can be further studied so as to develop a novel antimicrobial agent.

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