

EVALUATION OF ANTIBACTERIAL ACTIVITY OF ACETONE MACE EXTRACT (*Myristica fragrans* Houtt)

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Abstract

Antimicrobial activity of acetone mace extract was investigated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, using agar disc diffusion technique. Acetone mace extract was potentially effective with variable efficiency against the tested bacterial strains. Acetone mace extract can be used as natural alternative antibacterial agent against synthetic antimicrobial drug.

Key Words

Myristica fragrans, Antimicrobial potential, Disc diffusion mace, nutmeg

Introduction

Antimicrobial activity is the ability of a substance to inhibit or kill microbial cells. Profused use of synthetic antibiotics had led to the increasing number of resistant strains causing infections. Drug resistant strains are increasing in an alarming level and has led to the alternative antimicrobial agent.(1)

Plants are the most important source of phytochemicals. Phytochemicals are secondary metabolites which are rich in bioactive compounds such as alkaloids, flavonoids, quinones, phenolic compounds, tannins, terpenes and other chemical compounds.(2)

Medicinal aromatic plants and its phytochemical constituents are traditionally used as antimicrobial compounds.(3). Now owing to the drug resistance and side effects of the synthetic antibiotics, bioactive components of plant extracts are being explored for its antimicrobial property.(4)

Traditionally parts of plants like seed, leaf, bark are used as food ingredients. It has been found to be bactericidal and also possess broad spectrum activity.(5)

Pseudomonas aeruginosa, is an opportunistic bacterium that causes different conditions such as septicemia, pneumonia, urinary tract infection, endocarditis and skin-eye-ear infections. *Pseudomonas aeruginosa*, is one of the global threat of hospitalized infection. It is a difficult organism to control with antibiotics and disinfectants owing to low permeability of its cell wall and abundant resistance mechanism.(6)

Various studies have shown that most of the clinical isolates of *Staphylococcus aureus* are multiple drug resistant.(7) *Escherichia coli* is a pathogenic bacteria transmitted by infected food. Worldwide epidemics of this disease have been reported. The signs and symptoms of *E. coli* exposure include diarrhea, nausea, ulcerative colitis, abdominal pain and, in some cases, kidney disorders or death, especially among children(8)

Currently research is being carried out to investigate ethnobotanical uses of plants prevailing among native people(7). There are numerous reports evidencing the antimicrobial activity of plants against microorganisms.(9,10,)

Plants are important source of natural products with various biological properties. The presence of various phytochemicals especially flavonoids, polyphenols, phenolic acid, tannins, terpenes are known to be responsible for antimicrobial activities of these plant extracts.(11,12)

Nutmeg (*Myristica fragrans* Houtt) , is an ever green tree, native of Banda islands of Eastern Indonesia. In India , it is mainly cultivated in South India particularly in Kerala, Tamil Nadu and Karnataka. It belongs to the Myristicaceae family.(13). Mace is the aril part of the seed which constitutes the outermost third integument of the seed, covering its basal part by scarlet yellow ribbon like lobes, and is strongly aromatic in nature.(14)

Mace has been used in Indonesian folk medicine as aromatic stomachics, analgesics and a medicine for rheumatism(15) . Mace is traditionally used as a spice and flavouring agent in food. The use of spices in food has been a common practice since ancient times, they impart aroma, mask undesirable odours and can make food more pleasant and tastier. Spices are usually used as flavouring and coloring agents, and also used in food preservation for centuries as they are known to exert antioxidant and antimicrobial activity.(16,17)

The current study is an attempt to determine the antimicrobial activity of the acetone mace extract against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*.

Materials and Methods

Sample Extraction

The dried powder sample of mace (80g) was extracted three times by hot percolation method with 1:5 ratio volume of acetone at room temperature for 72 hrs. The filtrate so were concentrated under reduced pressure at 40 degree Celsius and stored at room temperature for use in subsequent experiments

Micro organisms

The bacterial spp. used for the test were *Staphylococcus aureus* (S. aureus), *Escherichia coli* (E. coli) and *Pseudomonas aeruginosa* (P. aeruginosa). All the stock cultures were obtained from Microlab, Saveetha Dental College, Chennai, Tamilnadu, India.

Inoculum preparation

Nutrient agar /broth (Himedia, India.) were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth (NA) at 37°C for 72 hrs.

Antimicrobial Activity

Antibacterial activity of acetone mace extract against three pathogenic bacteria were investigated by agar disc diffusion method. The extract was dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter and stored at 4°C. For the determination of zone of inhibition , pure microbial strains were taken and a standard antibiotic for comparison of results. The extract was screened for their antibacterial against the *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*. The set of five dilutions (10,25,50,100µg/ml) of acetone mace extract and standard drug were prepared in double distilled water using nutrient agar tubes. Mueller- Hilton sterile agar plates were seeded with indicator bacterial strain (10⁸cfu) and allowed to stay at 37°C for 3 hours. The zones of growth inhibition around

the disks were measured after 18 to 24 hours of incubation at 37°C for bacteria. The sensitivities of the microorganism species to the acetone mace extract were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8mm were considered as not active against microorganisms.

Statistical Analysis

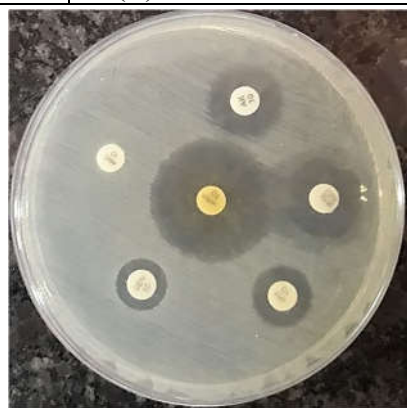
The data are given as the Mean $\pm$ SEM. The statistical analysis was performed by using one-way analysis of variance (ANOVA), followed by a Dunnett's multiple-comparison test with 95% confidence intervals. A P-value < 0.01 was considered to indicate a statistically significant difference.

Results

Table 1

Antibacterial activity of acetone mace extract (Mean $\pm$ SEM) (mm).

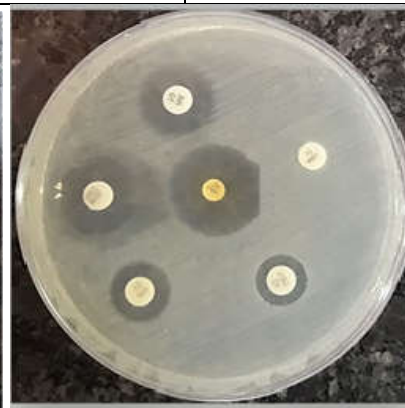
S. No	Conc (μ g/ml)	Zone of Inhibition (mm)		
		E.Coli	S.aureus	P.aeruginosa
	Acetone mace extract			
1	10(A)	3.04 $\pm$ 0.2	1.06 $\pm$ 0.74	3.06 $\pm$ 0.25
2	25(B)	5.12 $\pm$ 0.54	3.01 $\pm$ 0.24	5.03 $\pm$ 0.42
3	50(C)	6.89 $\pm$ 0.60	5.09 $\pm$ 0.45	7.05 $\pm$ 0.63
4	100(D)	11.54 $\pm$ 1.0	11.42 $\pm$ 1.0	10.54 $\pm$ 0.96
	Penicillin			
5	10(E)	14.56 $\pm$ 1.3	13.69 $\pm$ 1.2	14.09 $\pm$ 1.3



E.coli (fig1)



S.aureus(fig2)



P.aeruginosa(fig3)

The antibacterial activity of the acetone mace extract (Table1) was evident. In this study it was found that the MIC of acetone mace extract against *Staphylococcus aureus*, (fig2) *Escherichia coli*, (fig1) *Pseudomonas aeruginosa*, (fig3) was found to be 10 μ g/ml, 25 μ g/ml, 10 μ g/ml respectively. The antibacterial activity of acetone mace extract is comparable to standard antibiotic, penicillin.

Discussion

Phytochemical analysis of acetone mace extract reveals the presence of bioactive phytoconstituents(17). It is evident that the plant extract that is rich in tannins have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds(18), there by

killing the bacteria by directly damaging its cell membrane. Flavanoids are a group of phenolic compounds reported for their , antiviral and antimicrobial properties.(19).Alkaloids are also reported to possess antimicrobial activity.(20).

Acetone mace extract is rich in phenolic compounds, flavonoids, alkaloids and tannins, which might be responsible for its antibacterial property.

Conclusion

In conclusion, acetone mace extract exhibits antibacterial activity. Further studies are needed to isolate and characterize the bioactive principles to develop new antibacterial drug.

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