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STUDIES ON PHYTOCHEMICALS, SYNTHESIS OF SILVER NANOPARTICLES AND ANTIMICROBIAL ACTIVITY OF *Myristica fragrans* L.

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ABSTRACT

In the present study to investigate the phytochemicals, synthesis of silver nanoparticles and antimicrobial activity of *Myristica fragrans* L. The phytochemical like amino acids, alkaloids, flavonoids, saponins, steroids, and tannins were investigated whereas quantitative phytochemical *M. fragrans* like alkaloids, flavonoids, saponins, and terpenoids were determined respectively. The antimicrobial activity of *M. fragrans* with different concentration were the higher concentration of *M. fragrans* 100 mg was excellent biological activities when compared with low concentration of plant extract. The Proteus vulgaris (18mm) at maximum potential and moderate activity *Pseudomonas auroginosa* (18mm) recorded respectively. The antifungal properties of *M. fragrans* against some fungi were treated. The specific activity against *A. niger* (25mm) *Penicillum* sp. (25mm) at 100 mg of plant extract were observed respectively. The minimum antibacterial activity was 4mm zone of inhibition against *A. terreus* fungi tested respectively. The effect of silver nanoparticle synthesis by *M .fragrans* was more suitable for antimicrobial activities observed. the 100 mg of *M. fragrans* sample of silver nanoparticles were extract activities against B.cereus (14mm), *E.coli*, (25mm), *P. vulgaris* (8mm) *P. auroginosa* (28mm) and *S. aureus* (23mm) recorded respectively when compared with low concentration of *M. fragrans* synthesed silver nano particles synthesized by *M. fragrans* against some fungi like *A. flavus*, *A. terreus*, *A. niger*, *Fusarium* sp. and *Pencillium* sp. Treated.

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INTRODUCTION

The Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Over one and a half million practitioners of the Indian system of medicine use medicinal plants in preventive and curative applications. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as source of medicinal agents. The World Health Organization (WHO) has given guidelines to the member states to ensure about genuine use of plants and their parts before their use for human health Anonymous General guidelines for methodologies for Research and evaluation of traditional medicine, Anonymous Genenva, World Health Organization, (2000).

Nanoscience has been established recently as a new interdisciplinary science. It can be defined as a whole knowledge on fundamental properties of nano-size objects (Sergeev and Shabatina, 2008). The prefix 'nano' indicates one billionth or 109 units. The nature of this unit being determined by the word that follows. It is widely accepted in the context of nanoscience and nanotechnologies, the units should only be those of dimensions, rather than of any other unit of scientific measurement. It is widely agreed that nanoparticles are clusters of atoms in the size range of 1–100 nm. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology (Williams, 2008). In the present study to investigate the phytochemicals, synthesis of silver nanoparticles and antimicrobial activity of *Myristica fragrans* L.

MATERIALS AND METHODS

Collection of plant materials

The plant seed materials of *Myristica fragrans* seed were collected from the Thanjavur District, Tamil Nadu.

Preparation of *Myristica fragrans* seed power

The collected seed sample were air – dried. After air dried the samples was ground in grinding machine made for the laboratory. Exposure direct sunlight and avoided to prevent the loss of active components. These powdered materials were used for further analysis

Analysis of proximate content of *Myristica fragrans* seed

Determination of moisture (James 1995). Determination of Crude Fiber (CF). Determination of nitrogen free extracts (NFE). Acid Soluble and Insoluble Ash. Determination of Ash.

Preparation of seed extract

Phytochemical test were carried out on the. Aqueous. The extract *M. fragrans* seed powder using standard procedures for determination the phytochemical constituents as described by Sofowara (1993) Treas and Evans (1989) and Harborne (1973).

Qualitative phytochemical analysis

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by Brain and Turner (1975) and Evans (1996)

Quantitative phytochemical analysis

Alkaloid determination by using Harborne (1973) method. Estimation of flavonoids (Krishnah *et al*., 2009). Estimation of tannins (Van – Burden and Robinson, 1981). Estimation of total phenols

Antimicrobial activity

Determination of antimicrobial activity (Perez et al., 1990). Preparation of silver nitrate solution (Jebakumar et al., 2012)

RESULT

The proximate analysis of *M. fragrans* were analyzed and proximate in (Table -1). The crude fiber content of *M. fragrans* was maximum 0.25 % compared to other proximate tested. Similarly its lowered content of Ash (0.11%) was observed its powered of *M, fragrans*. The moisture and fat content of *M. fragrans* were (0.34 %, 1.24 %) respectively (Table – 1, Fig – 1).

In this investigation, the plant *Myristic a fragrans* seed extract which contain some of quantitative phytochemicals such as amino acids alkaloids, flavonoids, phenols, reducing sugar , saponnins, steroids, tannins, and terpenoids were tested. Among the investigation some of the bioactive chemicals like amino acids, alkaloids, flavonoids, saponins,steroids, and tannins were represented from the plant *Myristica fragrans* with aqueous extract whereas quantitatively analysis of phytochemicals such as alkaloids, flavonoids, saponins, and terpenoids was 0.58,0.45,0.84 and 089 mg / ml estimated from the plant *Myristica fragrans* (Table – 2 and 3, Plate - 1).

The investigation of antimicrobial properties of *M. fragrans* seed extract has maximum potential properties possess from the analysis the effect of antibacterial activity of *M. fragrans* with different concentration of 25, 50, 75 and 100µl treated against *Bacillus cereus, E.coli, Pseudomonas aeruginosa, P.vulgaris*, and *Staphyloccous aureus* were tested. The *Bacillus cereus* with suppression was 9, 10, 12 and 21mm zone of growth inhibition from *in*

vitro studies of effect of *Myristica fragrans* of different concentration of 25, 50, 75 and 100 µl were analyzed.

Whereas *E.coli* bacteria was 13, 14, 16 and 22 µl zone of inhibition from the plant extract of 25,50, 75, 100 µl of *Myristica fragrans* seed extract recorded respectively . In the case of clinical bacteria *Pseudomonas auroginosa* was highly suppressed such as 03, 07, 17 and 18 µl from the plant extract of 25, 50, 75, and 100 µl treated respectively. The bacteria *P. vulgris* was 04, 6, 12 and 18 µl zone of growth inhibition observed with respective respectively. The clinically important bacteria *Staphyloccous aureus* was 9, 10, 15 and 18 µl zone formation for the growth suppression from plant *M. fragrans* seed extract (Table – 4, Plate – 2, Fig - 2).

Studies on the efficacy of antifungal activity of *M. fragrans* seed extract of various concentration of 25, 50, 75 and 100 mm treated with *Aspergillus flavus* was 8, 9, 10 and 12 µl zone of measuring for growth control with observed respectively. The same genus of *Aspergillus niger* was 20, 22, 25 and 28 µl zone of inhibition from the concentration of 25, 50, 75 and 7, 4, 8 and 5 µl of lesser zone of inhibition observed from the respective concentration of test plant. The great fungi *Fusarium* sp was 12, 15, 17 and 22 µl measured from the various treatment of 25, 50, 75 and 100 µl of *Myristica frgrans* seed extract recorded respectively. In the case of *penicillium* sp was 11 , 15, 18 and 25 mm of controlling the fungi the treatment of 25, 50, 75 and 100 µl of test plant extract represented respectively (Table - 5, Plate - 3, Fig - 3).

Medicinal plants represent rich source of antimicrobial agents. But recently scientific form some of the advanced nanotechnology for controlling the microbes were invest iced. From this analysis, the determination effect silver nano particles from *Myristica fragrans* seed extract plant extract was more potential when compared to crude extract of medicinal plants. In that scence, the silver nanoparticles of 25, 50, 75 and 100 µl of condensed materials against bacteria *Baciiius cereus* was 8, 10, 13 and 14 µl zone of inhibition observed respectively whereas *E.coli*, was 3, 5, 8 and 25 µl zone f inhibition and growth reducing from the nanoparicles were conformed with respective plant nanoparticles. The bacteria *Proteus valgarus* was 4, 6, 8 and 10 µl zone of inhibition observed with respective concentration of 25, 50, 75 and 100 µl of silver nano particles analyzed whereas *Pseudomons auroginosa* was also 6, 9, 11, and 18, mm zone inhibition very greatly observed with 25, 50, 75 and 100 µl of nano particles synthesesed by plant system respectively. The clinical isolates of *Stphylococcus aureus* was 8, 10, 16, and 23 µl of zone minimized suppression but the function of nano particle was highly responsible and immunized process respectively (Table - 6, Plate -4, Fig - 4).

According to the silver nano particle from the *Myristica fragrans* has more potential against some fungi such as *Aspergillus flavus A.niger* , *A.terreus* , *Fusarium* sp, and *Penicillium* sp treated. The effect of silver nano particles of 25, 50, 75 and 100 µl treated with *Aspergillus flavus* was 8, 12, 14 and 25mm zone of inhibition observed respectively whereas *A. niger* was 7, 5, 9 and 10 µl zone from the various concentration of 25, 50, 75, and 100 µl treated respectively. In the case of *A.terreus* was 8, 13, 15, and 25 µl zone of growth measured from the treatment of 25, 50, 75 and 100 µl of medicinal plant synthesized silver nanoparticles respectively. The fungi *Fusarium* sp. was 5, 7, 10, and 11 µl zone of growth suppression from the respective plant silver nanoparticles respectively. According to the *Penicillium* sp. was 7, 8, 10, and 12 µl potentially suppressed from the silver nanoparticles also primate the increase the zone of inhibition so the silver nanoparticles was better for antimicrobial potential when compared with medicinal plant crude extract treatment (Table - 7, Plate - 5, Fig - 5).

DISCUSSION

In the present investigation suggested that the entitled on –Studies on Phytochemicals, synthesis of silvernanoparticles and antimicrobial activity of *Myristica fragrans* L. were determinate. The estimation of qualitative phytochemical compnds of *Myristic fragrans*. It was amino acid, alkaloids, flavonoids, phenols, reducing sugar, saponnins, tannins and terpenoids tested. The test plant has maximum extract and tannins identified whereas quantity analysis the phytochemicals such as 058, 045, 084, and 089 mg/ g with alkaloids, flavonoids, saponnis and terpenoid recorded respectively. This bioactive compounds which responsible for the biological activities including nano technology.

Proximate content were carried out of the selected spice to know the nutritional significance of these frequently consumed species in the traditional medicines. These analyses revealed some interesting findings. Ash content was found to be low (4.44 ± 0.11 mg/g) when compared to the fibre, moisture and fat content in nutmeg. As a nutritive value of food, fibers in the diet are necessary for digestion and for effective elimination of wastes, and can lower the serum cholesterol,

the risk of coronary heart disease, hypertension, constipation, diabetes, and colon and breast cancer Hussain *et al*., (2009). Intake of such medicinal plants in traditional recipes showed evidence that dietary fiber is associated with enhanced insulin sensitivity and therefore may have a role in the prevention and control of Type 2 diabetes Vadivel *et al*., (2005). Medicinal plants can be considered as a valuable source of dietary fiber in human nutrition.

Phytochemical is a natural bioactive compound found in plants such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease. Photochemical are divided into two groups which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds (Krishnaiah *et al*., 2007) and many more such as flavonoids, tannins and soon.

The antimicrobial activity of the ethanol extracts of *J. curcas* and *M. frangrans* against pathogenic microorganisms isolated from cutting edge of the barber‘s clipper are presented in Figures 2 and 3. Ethanol extract of the seeds exhibited higher antimicrobial activity against all the test organisms than the aqueous. However, the reference drug had higher antimicrobial activity than the ethanol extracts of the two plant seeds against all test organisms. Ethanol extract of *J. curcas* had higher antimicrobial activity against *S. aureus* (15 mm), *A. niger* (13.5 mm) and *P. fellatum* (9.5 mm) than the ethanol extract of *M. frangrans* which had higher antimicrobial activity on the other two test organisms *(E. coli* and *S. pyogenes*) than that of *J. curcas* activity against test isolates at concentrations up to 50 mg/ml and above with the exception of *P. fellutanum* and *A. niger* with MIC at 100 mg/ml. The aqueous extract of *J. curcas* seed had higher antimicrobial activity against *A. niger* (14.5 mm), *S. aureus* (14 mm) and *Lactobacillus* sp. (13 mm) amongst other test isolates. The highest antimicrobial activity (13.5 mm) of aqueous extract of *M. frangrans* was recorded against *A. niger* as shown. The known pathogenic microorganisms isolated from cutting edge of the barber‘s clipper are similar to that reported by Adamu *et al*., (2012).

In the current research stated that the antibacterial activities of *Myristica fragrans* against some bacteria such as *Bacillus cereus, E coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Staphylococcus aureus* represented respectively. In current research stated that the antifungal properties of medicinal plant with various concentrations against some fungi treated. The higher concentration of *Myristica fragrans* were highly suppressive ties can be identified.

Table 1: Analysis of proximate content of *M. fragrans* seed

Proximate content	Quantity (%)
Ash	0.11
Crude Fiber	0.25
Moisture	0.34
Fat	1.24
N Free Extractives (NFE)	2.10

Table 2: Qualitative analysis of phytochemical compounds of *M. fragrans* plant

Phytochemical compounds	Aqueous
Amino acids	+
Alkaloids	+
Flavonoids	+
Phenols	-
Reducing sugar	-
Saponnins	+
Steroids	+
Tannins	+
Terpenoids	+

(+) – present (-) – absent

Table 3: Quantitative analysis of phytochemical compounds of *M. fragrans* plant

Phytochemical compounds	Quantity (mg/ml)
Alkaloids	0.58
Flavonoids	0.45
Saponnins	0.84
Terpenoids	0.89

Table 4: Studies on the effect of antibacterial activity of *M. fragrans* against bacteria

Name of bacteria	Zone of inhibition (mm)			
	25 mg	50 mg	75 mg	100 mg
Bacillus cereus	9	10	12	21
E. coli	13	14	16	22
Pseudomonas aeruginosa	3	7	17	18
Proteus vulgaris	4	6	12	18
Staphylococcus aureus	9	10	15	18

Table 5: Studies on the effect of antifungal activity of *M. fragrans* against fungi

Name of fungi	Zone of inhibition (mm)			
	25 mg	50 mg	75 mg	100 mg
Aspersillus flavus	8	9	10	12
A.niger	20	22	25	28
A. terreus	4	6	8	10
Fusarium sp.	12	15	17	22
Penicillum sp.	11	15	18	25

Table 6: Determination of effect of silver nanoparticles from *M. fragrans* against some bacteria

Name of bacteria	Zone of inhibition (mm)			
	25 mg	50 mg	75 mg	100 mg
Bacillus cereus	8	10	13	14
E. coli	3	5	8	25
Pseudomonas areginosa	6	9	11	18
Proteus vulgaris	4	6	8	10
Staphylococcus aureus	8	10	16	23

Table 7: Determination of effect of silver nanoparticles from *M. fragrans* against some fungi

Name of fungi	Zone of inhibition(mm)			
	25 mg	50 mg	75 mg	100 mg
Aspersillus flavus	8	12	14	25
A. niger	7	5	9	10
A. terreus	8	13	15	25
Fusarium sp.	5	7	10	11
Penicillum sp.	7	8	10	12

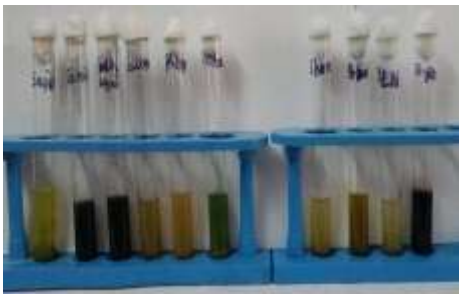

Plate 1: Qualitative analysis of phytochemical compounds of *M. fragrans*

Bacillus cereus ***E. coli*** ***P. aeruginosa*** ***P. vulgaris*** ***Staph. aureus***

A – 25 mg, B – 50 mg, C – 75 mg, D – 100 mg

Plate 2: Studies on the effect of antibacterial activity of *M. fragrans* against bacteria

Aspersillus flavus ***A. niger*** ***A. terreus*** ***Fusarium sp.*** ***Penicillum sp.***

A – 25 mg, B – 50 mg, C – 75 mg, D – 100 mg

Plate 3: Studies on the effect of antifungal activity of *M. fragrans* against fungi

Bacillus cereus ***E. coli*** ***P. vulgaris*** ***P. aeruginosa*** ***Staph. aureus***

A – 25 mg, B – 50 mg, C – 75 mg, D – 100 mg

Plate 4: Determination of effect of silver nanoparticles from *M. fragrans* against some bacteria

Aspersillus flavus ***A. niger*** ***A. terreus*** ***Fusarium sp.*** ***Penicillum sp.***

A – 25 mg, B – 50 mg, C – 75 mg, D – 100 mg

Plate 5: Determination of effect of silver nanoparticles from *M. fragrans* against some fungi

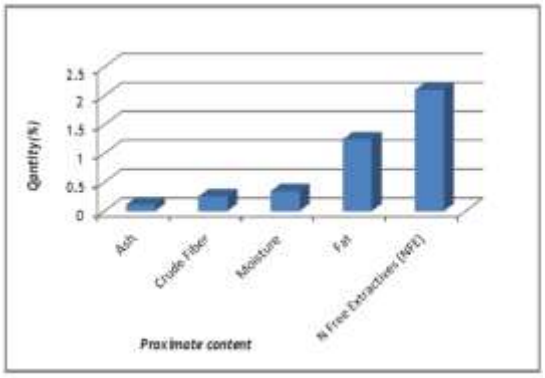

Fig 1: Analysis of proximate content of *M. fragrans*

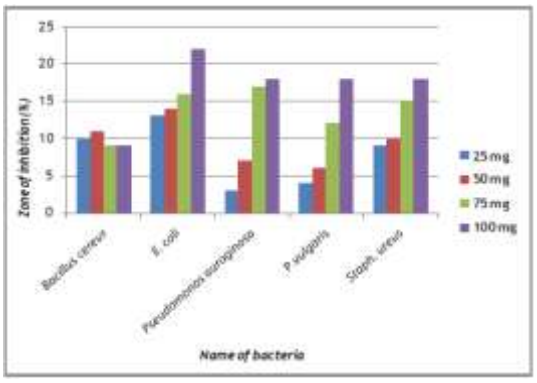

Fig 2: Studies on the effect of antibacterial activity of *M. fragrans* against bacteria

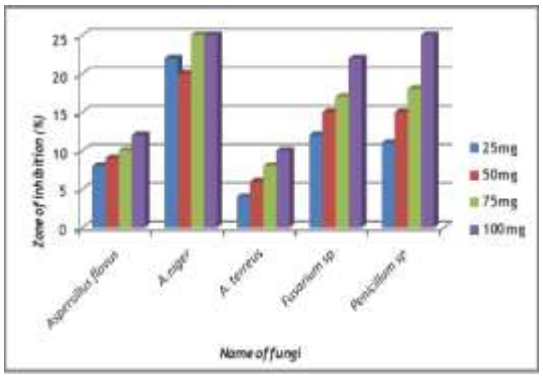

Fig 3: Studies on the effect of antifungal activity of *M. fragrans* against fungi

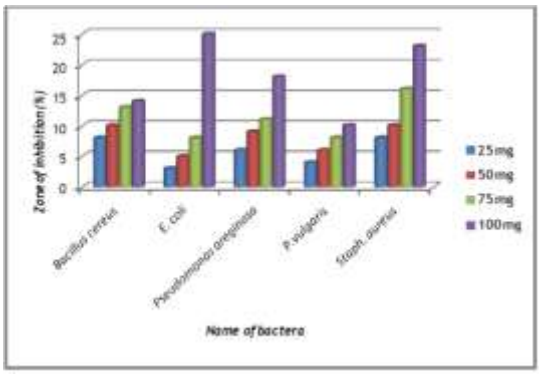

Fig 4: Determination of effect of silver nanoparticles from M. fragrans against some bacteria

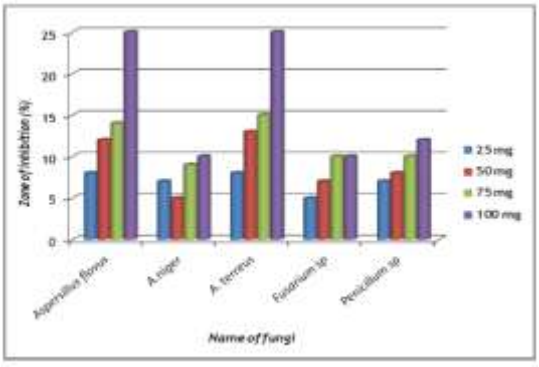

Fig 5: Determination of effect of silver nanoparticles from M. fragrans against some fungi

CONCLUSION

The present study exhibit a simple environmentally benign method of synthesis of silver nanoparticles from a novel primitive plant source. This method can be further used for industrial production of nanoparticles at room temperature and with a single step. Since the nanoparticles thus synthesized shows antimicrobial activity, they can be used in various fields such as pharmaceutical industry and so on. Silver nanoparticles might be useful for the development of newer and more potent antimicrobial agents. The data represented in our study contribute to a novel and unexplored area of nanomaterials as alternative medicine.

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