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Myristica fragrans (Nutmeg) OIL MEDIATED SILVER NANOPARTICLE SYNTHESIS, CHARACTERISATION AND ITS ANTIMICROBIAL ASSESSMENT

M. CHRISTINA PAULINE[1], R. SANGEETHA[1], M. MANIKANDAN[1], PREMRAJ LOGANATHAN[1] AND J. M. V. KALAIARASI[1*]

[1]PG and Research Department of Advanced Zoology and Biotechnology, Loyola College, University of Madras, Chennai, Tamil Nadu, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Nanotechnology is significantly influencing science and economy in 21st century. Especially, plant mediated synthesis of nanoparticle is a green approach that interconnects nanotechnology and plant biotechnology. Whereby, Silver (Ag) nanoparticles (NPs) were prepared by a biological procedure, where, the procured essential oil of Nutmeg was used as a capper and reductant agent. Characterisation of the Nutmeg NPs was carried out using UV -visible spectroscopy, Fourier transformed infrared spectroscopy (FT -IR), Scanning electron microscopy (SEM). These NPs were utilized as an effective anti-microbial and anti-fungal agent in different range. Moreover, the isolation and recovery of Nutmeg NPs was efficient and cost effective.

Keywords: Essential oil; nutmeg- *Myristica fragrans*; Ag NPs; characterization; antibacterial and antifungal.

1. INTRODUCTION

Nanotechnology is a rapidly growing area of scientific interest due to its wide range of applications. It is quite surprising, that from simple microbes and bacteria up to more evolved plants, its possible to get nanoparticles. Nanomaterials and Nanoparticles are efficiently used in the field of medicine for the purpose of drug delivery, diagnosis and treatment of some disease, as well as development of anti-bacterial, anti-fungal agents [1].

Silver is a common metal used for synthesis of nanoparticles, which was accepted for multi-purpose applications. Nanoparticle synthesized via physical and chemical methods are highly expensive and toxic to the environment. Hence, a cost effective, rapid, easy, eco-friendly and non-toxic alternative methods of bio-synthesis of nanoparticles is the need of the hour [2].

Plants and essential oils contain lots of functional chemical compounds, which can be used as a good stabilizing and reducing agent during nanoparticles synthesis. Essential oils are also used as functional ingredients in food, cosmetics, drinks, toiletries, etc., [3].

Essential oils play an important role in their own ways. *Myristica fragrans* plays an important role in terms of its medicinal and aromatic value, which belongs to the family Myristicaceae. Nutmeg has been

**Corresponding author: Email: dr.kalaiarasi@yahoo.co.in;*

widely used in the traditional medicine, and as flavoring agents in the food.

The biological activities of the essential oils are correlated to the presence of specific chemical compound. Essential oil and their components, are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use [4].

Most of the researchers synthesizing nanoparticles by using bacteria, yeast, fungi and various part of the plants, still a few of them are using essential oils. In this [5] present study we report the synthesis of silver nanoparticle by using Nutmeg essential oil, as reducing agent while silver nitrate acts as a precursor. The structural properties of synthesized Ag Nps have been confirmed by using UV- Vis, FT-IR (Fourier Transform Infrared Spectroscopy), SEM (Scanning Electron Microscopy) techniques and anti-bacterial and anti-fungal activities were examined.

2. MATERIALS AND METHODS

2.1 Sample Collection

The *Myristica fragrans* (Nutmeg) oil was obtained from TEGRAJ & CO, a government recognized (TNGST 030223), aromatic oil stores and stored in dark condition until use.

2.2 Synthesis of Nanoparticles

1mM Silver nitrate aqueous solution was prepared. Then, 100ml of Nutmeg oil was added to 100ml of 1mM silver nitrate solution. Nutmeg oil was used as reductant for silver nanoparticle synthesis and kept in the shaker at 120 rpm in room temperature, under dark condition. After colour change, centrifugation was done and pellet was separated and washed several time with distilled water and dried for further investigations.

2.3 Characterisation of Silver Nanoparticles

The colour change in the reaction mixture (silver Nitrate + Nutmeg oil) was seized through visual observation. The synthesized silver nanoparticles were characterized by different techniques. The UV-Vis analysis spectrum, was recorded in ELIZA reader over 300-500 nm range. FT-IR analysis of the dried AgNps was carried out by KBr pellet method and the presence of various vibrational modes in the synthesized nanoparticles, was investigated. The SEM technique was employed to visualize the size and morphology of the particles.

2.4 Anti-microbial Activity

Well diffusion technique [6] was used to evaluate the antimicrobial property of synthesized AgNps of Nutmeg oil, against pathogenic bacterial strains *staphylococcus aureus* MTCC-98 (Gram positive), *Klebsiella pneumoniea* MTCC-7407, *Pseudomonas aeruginosa* MTCC-7436, *Salmonella typhi* MTCC-98, as well as fungal strains *Aspergillus niger*, MTCC 183, *Apergillus flavus*, MTCC-9390. *Mucor sps,* and *Fusarium solani* MTCC 184. Muller Hington Agar was used, for bacterial cultures and Potato Dextrose Agar, for fungal cultures. The plates were seeded with respective microbial strains and incubated. The zone of inhibition was measured.

3. RESULTS AND DISCUSSION

3.1 Synthesis of Silver Nanoparticles

3.1.1 UV-spectrometry analysis

Filtered (nutmeg) *Myristica fragrans* oil with silver nitrate at 1 mM, showed a change in color, from off-white to greyish black (Fig. 1). This is due to the excitation of surface plasmon resonance effect by AgNPs. Silver nanoparticles have free electrons, which give rise to surface plasmon resonance absorption band, because of combined vibration of electrons of element nanoparticles in resonance with the light wave. The reduction of AgNP in the (nutmeg) *M. fragrans* solution of the silver complex during the reaction, was confirmed by the UV-visible spectra. The AgNPS solution and (nutmeg) *M. fragrans* oil, did not show any peak between 300 nm and 399 nm. AgNPs synthesis was evaluated under UV-visible spectroscopy at different period (1 hour – 24 hours) (Graph 1), in order to examine the stability of the formed nanoparticles. It was recorded, that with increased contact time, the peak was narrow and sharper at 420 nm at final allotted period. The sharper peak indicated the formation of mono disperse nanoparticles from the oil of (nutmeg) *M. fragrans*. The surface plasmon resonance (SPR) peak centered near 420 is mere close to that reported by [7] affirm the reduction of $Ag+$ to Ag^{o}.

3.2 FTIR Analysis

FTIR spectroscopy reveals the presence of chemical composition and functional groups in (nutmeg) *M. fragrans* silver nanoparticles. The FTIR spectrum of silver nanoparticle is shown in Graph 2. Four main bands could be observed from the figure. The broad

band appearing at 3610 cm^{-1} is assigned for N-H stretching vibration, indicating the presence of secondary amine. The strong intense peaks at 3387 and 3332 cm^{-1} correspond to O-H and C-H stretch vibrations, as Alkanes have no functional groups. Their IR spectrum displays only C-C and C-H bond vibrations. Of these the most useful are the C-H bands, which appear around 3000 cm^{-1}. Since most organic molecules have such bonds, which they display in their spectrum. The result of this FTIR spectroscopic study, confirmed, that the (nutmeg) *M. fragrans* has the ability to perform dual functions of decline and stabilization of silver nanoparticles. According to [8] the results confirm the presence of phenols and proteins which may act as reducing and stabilizing agents for silver nanoparticles.

3.3 Scanning Electron Microscopy

In scanning electron microscopy (SEM) analysis, the morphology of synthesized AgNPs using (nutmeg) *M. fragrans* oil was observed, in which, silver nanoparticles were in globular form (Fig. 2). The results shown below proved, that, the nanoparticles are produced due to the action of (nutmeg) *M. fragrans* essential oil, which play as good biomedical application to kill pathogenic microbes. The SEM confirmed *M. fragrans,* synthesized nanoparticles sized from 33.2 nm to 39.7 nm (Fig. 2) [8]. State that the size of the nanoparticle is determined by the interaction with the surface membrane, smaller their size greater their capability.

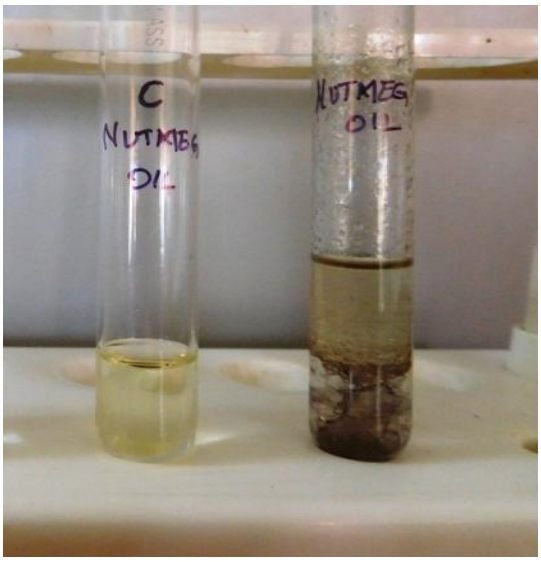
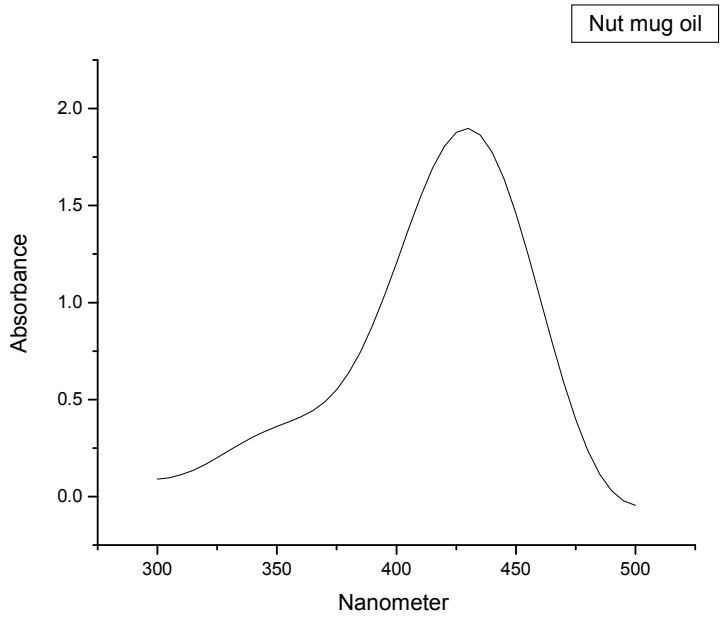


Fig. 1. Colour change from off – white to greyish black

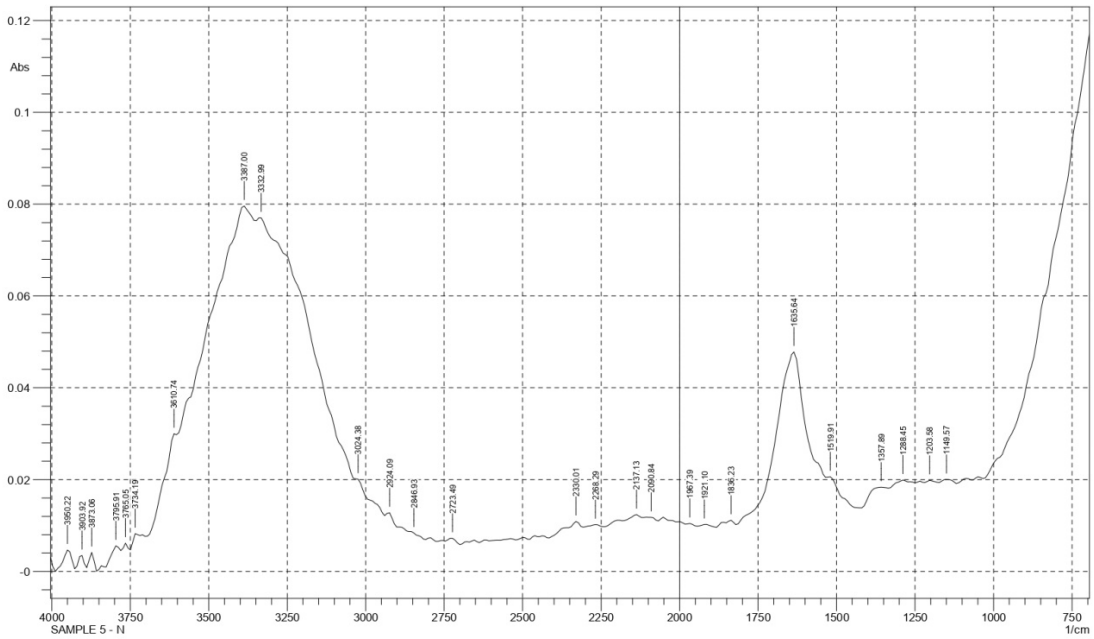
3.4 Antimicrobial Activity

Myristica fragrans oil showed the effective antimicrobial activity, against various pathogens, and high inhibition zone was shown in Graph 3 and Graph 4. The essential oil of *Myristica fragrans* showed least inhibition against *S.typhii*. All other bacteria and fungi were observed to be sensitive [5]; to the *Myristica fragrans* synthesized silver nanoparticles.



Graph 1. UV-visible spectroscopy

Myristica fragrans is mostly compared to the standard antibacterial and antifungal reference gentamycin and clotrimazole [6], respectively to some extent. Similar results were observed by [9], where this nutmeg mediated silver nanoparticle reveals a strong dose dependent activity [10].



Graph 2. FTIR spectrum of Nutmeg silver nanoparticle

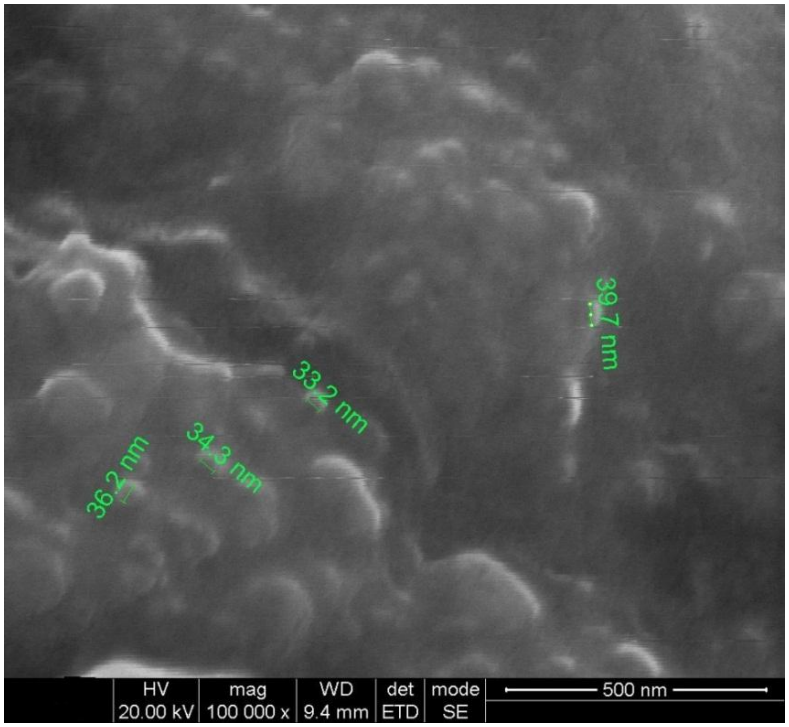
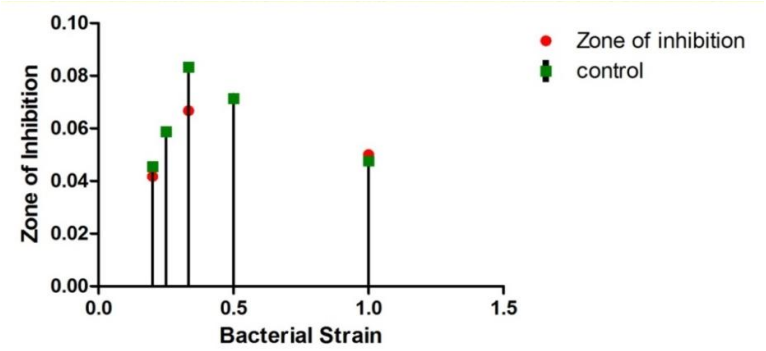
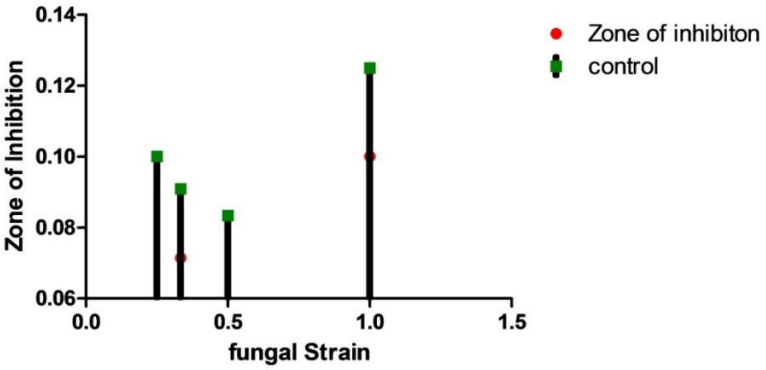


Fig. 2. SEM image of synthesized AgNPs using (nutmeg) *M. fragrans* oil



Graph 3. Antibacterial activity of synthesized (nutmeg) *M. fragrans* oil silver nanoparticles



Graph 4. Antifungal activity of synthesized (nutmeg) *M. fragrans* oil silver nanoparticles

4. CONCLUSION

In the conclusions, for the first time, we report green synthesis of AgNps using *Myristica fragrans* (Nutmeg oil) in biological method. It is much safe, ecofriendly and cost effective when compared to the chemical synthesis. The Nutmeg oil, act as a good stabilizing and reducing agents. The silver nanoparticles synthesized, were confirmed by UV, FTIR, and SEM revealed that the average particle size is 32 nm.

The effectiveness of antibacterial and antifungal activity of biosynthesized AgNps, using *Myristica fragrans,* against pathogenic microbes were examined, which reveal significant antibacterial, antifungal activity against various multidrug resistance microbes. In particular, these nanoparticles exhibited greater antibacterial effect towards *S.aureus,* when compared to others, in antifungal activity against *A. niger.* So finally we conclude that a Nutmeg oil mediated silver nanoparticles has a good antimicrobial effect.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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