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GREEN NANOSILVER: A POTENT ANTIFUNGAL FOR URINARY TRACT INFECTIONS

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ABSTRACT

Recent researches in the field of life science and drug discovery leads to affirmations regarding the application of nanotechnology in drug development process. There are so many applications of nanotechnology in the diverse fields including physical science; medical science etc. Current study discussed the safe and friendly synthesis of nanoparticle using metal silver. Silver has its own importance in therapies mentioned in Ayurvedas and traditional Indian medicines. Urinary tract infection especially nosocomial infections play a crucial role in national health caused by uropathogens includes fungal and bacterial infections. Study reports the antifungal activity of green synthesized nanoparticles using *Carica papaya* leaves using traditional microbiological methods. Facts founds in the study, compared with the traditional antifungal drugs which shows that synthesized silver nanoparticles with the extract having more potential antifungal activity.

KEYWORDS

Silver nanoparticles, Urinary tract infection and Antifungal.

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INTRODUCTION

Commonest universal problem in the infectious disease therapies are antibiotic resistance. Researchers are in the race to find and generate newer and newer restriction strategies. Urinary tract infection caused by the fungal pathogens is one of the most challenging tasks to manage with. Current antifungal are available in market are at high risk regarding the new adaptation in fungus^{1,2}. Recently, there are more updates from herbal drug

development in developing countries; various formulations have been reported in drug development from plant. A survey of literature reveals, there are baskets of active pharma compounds available in selected plant in this study named as *Carica papaya*. Various studies were done on this plant and reported activities from every nook of world. Still there are some compounds which are available, delivered, applied but unable to reach at target site. Hence it's a first report of study focused on the development of the silver nanoparticle using same plant leaves and application of methanolic extract of same plant as a potential antifungal agent mechanism of action is hypothesized in the present study³⁻⁵.

MATERIALS AND METHODS

Preparation of plant extract

The well-known plant leaves called as *Carica papaya* were included in this study. Fresh green plant leaves were collected. Primarily the leaves were washed with tap water and then with Distilled water. Washed cleaned leaves were dried with water absorbent. Leaves were then chopped finely with knife and dispensed in 100 ml of sterile deionised distilled water and boiled for one - two hour at 82°C. Then the leaf extracts were collected in separate conical flasks by standard filtration method.

Preparation of Silver nanoparticles

10⁻³ M Silver nitrate solution was prepared and stored in brown bottles. 5ml of leaf extracts was taken in Biochemical Oxygen Demand (BOD) bottle separately and to this 100ml of silver nitrate solution was added. The colour change of the leaf extracts from pale yellow to green and reddish was checked periodically. Then it was incubated at room temperature for further incubation till 28 hours. The colour change indicated that the silver nano particles were synthesized from the leaves and centrifuged at 9000 rpm for 30 minutes where pellets used for antifungal activity⁶.

Characterization of Silver nanoparticles

Ultra Violet -vis spectra analysis

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the

UV-vis spectrum of the PerkinElmer spectrophotometer at a resolution of 1 nm (from 300 to 600 nm) in 2 ml quartz cuvette with 1 cm path length.

Scanning Electron Microscopy analysis

The Morphological characterization of the samples was done using Scanning Electron Microscopy analysis. The samples were dispersed on a slide and then coated with platinum in an auto fine coater. After that the material was subjected to analysis.

Fourier transforms infrared spectroscopy (FTIR) analysis

The characterization of functional groups on the surface of Silver nanoparticles by plant extracts were investigated by Fourier transform infrared spectroscopy analysis (Shimadzu) and the spectra was scanned in the range of 4000-400 cm⁻¹ range at a resolution of 4 cm⁻¹. The sample were prepared by dispersing the silver nanoparticles uniformly in a matrix of dry potassium bromide, compressed to form an almost transparent disc. Potassium bromide was used as a standard analyse the samples.

Screening of Antifungal activity of plant extract

Sabouraud Dextrose Agar (SDA) medium was prepared according to the manufacturer's instructions by dissolving 65 gm of the Sabouraud Dextrose Agar in 1000 ml of distilled water then shaken, heated and autoclaved. This medium was poured aseptically at 45°C into sterilized Petri plates with the aid of sterile pipette of 20 ml capacity on the flat horizontal surface to a depth of 20 mm. After complete solidification, 3 wells were made aseptically with a diameter of 6 mm on the surface of each agar plate. One containing antibiotic and one with silver nanoparticle and control. Different concentration used in dilution 25, 50µl. Microbial Type Culture Collection strains of fungus were already stocked for *Candida albicans* (Microbial Type Culture Collection 227), *A. niger* (Microbial Type Culture Collection 1344) *A. fumigates* (Microbial Type Culture Collection 9657). Later, a sterile cotton swab was dipped into the fungal suspension and the surplus was removed by rotating the swab to the sides of the test tube used. The Sabouraud Dextrose Agar media were inoculated by

even streaking of the swab over the entire surface of each plate⁷.

RESULTS

Biosynthesis of Silver nanoparticles using *Carica papaya* leaves

Leaves of *Carica papaya* plant is a very well-known source for flavonoids and phenolics. Flavonoids play an important role in the reduction process for biosynthesis of silver nanoparticles. Accordingly, the high content source of flavonoids and phenolic acids in plant leaves extract supports the potential bio reduction of Ag^+ to Ag^0 .

Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the colour change (Figure No.1). The colour change is due to the Surface Plasmon Resonance action. There are metal nanoparticles containing free electrons, which give the absorption band, due to the similar vibration of electrons of metal nanoparticles in resonance with light wave.

Characterization of Silver nanoparticles

UV spectra Analysis

The colour of the solution changed from pale yellow to dark green. The sharp clear intense bands of silver nanoparticles were observed at 408nm. Initially the solution colour was light yellowish than with time duration it turned from yellowish to light brown. It is observed silver nanoparticles have a distinguished colour in aqueous solution because of the surface plasmon resonance in silver nanoparticles. The metal (silver) nanoparticles have free electrons, which are responsible for the Surface Plasmon Resonance absorption band. Various literatures have reported the 410-420nm (Figure No.2).

Scanning Electron Microscopy Analysis

Scanning Electron Microscopy analysis provided the morphology and size details of the nanoparticles. Results shows high density Silver nanoparticles synthesized by the plant extract of *Carica papaya* more confirmed the presence of silver nanoparticles the shape of the silver nanoparticles found spherical and hexagonal in some places which looks adhered and clumps. Size may be ranges from 12nms-15nm (Figure No.3).

Fourier transforms infrared spectroscopy analysis

Spectrum was analysed for identification of different biomolecules adsorbed on the surface of nanoparticles, and also to find out their role in reduction and stabilizing the nanoparticles. The FTIR spectrum of synthesized silver nanoparticles peaks were observed at $3905.30, 3759\text{cm}^{-1}, 827\text{cm}^{-1}$ which are associated OH stretching, C=C stretching, CH stretching, CH stretching respectively. $1599, 1370\text{cm}^{-1}$ are associated with nitro groups C=N stretching, C=N stretching, N-H stretching, CH stretching, CN stretching, C-Cl stretching. In the synthesized Silver nanoparticles from papaya leaves peaks were observed at 3439.72cm^{-1} , and above correspond to O-H groups, H bonded alcohols and phenols, still some flavonoids are present in the extract which supported by Gas Chromatography and Mass Spectroscopy data. Conclusion can be made the capping of such carboxylic group and other functional group on silver nanoparticle (Figure No.4).

Antifungal activity of *Carica papaya* silver nanoparticles containing plant extract

In vitro antifungal activity of silver nanoparticles containing plant extract was studied with standard antibiotics. On the basis of this study, silver nanoparticles containing plant extract shows different activity on clinical isolates which was confirmed by measuring zone of inhibition as compare to standard antibiotic. In this study, *Candida albicans* and *Aspergillus fumigatus* was found most sensitive against silver nanoparticles containing plant extract and found more effective like antibiotic i.e. 11mm and 13mm. zone of inhibition. Shown in Table No.1 and Figure No.5.

DISCUSSION

Catheter Associated Urinary Tract Infection (CAUTI) is the one of the most deadly reason behind high mortality rate among the hospital acquired infections according to Rahul Mittal et al. 2009⁸, 40% Nosocomial infections spread due to unhygienic and wrong disease management. *Candida albicans* responsible for various site infections e.g. pelvis, ureter, kidneys and bladder,

and causes cystitis or pyelonephritis^{9,10}. Disease management is truly difficult in the candidiasis. The overall resistance in *Candida* species. To fluconazole and variconazole is considered to be around 3-6% and level of resistance has remained constant over a decade by pfaller and Yee chunet al.^{11,12}, Targeting the cell membrane and site of attachment in fungal infection is difficult. All over scenarios of fungal infection like *Aspergillus niger* and fumigates is also alarming. Women's are more prone to such an infections.

Mechanism of drug resistance is well known in fungal uropathogens, *Candida. albicans* and *Aspergillus* sp. both having efflux pump. There is a specific mechanism in the *Candida. albicans* in which it overexpress genes and regulates ABC transporters by which drug unable to enter in cell or efflux takes place. In candidiasis tissues get lesions and ruptured. It attaches to internal parenchymal walls of bladders^{13, 14}.

It is also known that indiscriminate use of antibiotic for prolong period develops such antibiotic resistance infections¹⁵.

Table No.1: Zone of inhibition (in mm) of fungal isolates at various concentrations (µl) of herbal nanoparticles extracts (extract +AgNp)

S.No	Test Clinical isolates	Concentration in 25 µl	Concentration in 50 µl	StandardAntibiotics
1	<i>Aspergillus Niger</i>	Not observed clear	9 mm	11mm
2	<i>Aspergillus fumigates</i>	4mm	11mm	6mm
3	<i>Candida albicans</i>	9mm	13mm	7mm



Figure No.1: Synthesis of silver nanoparticle using *c.papaya* leaf

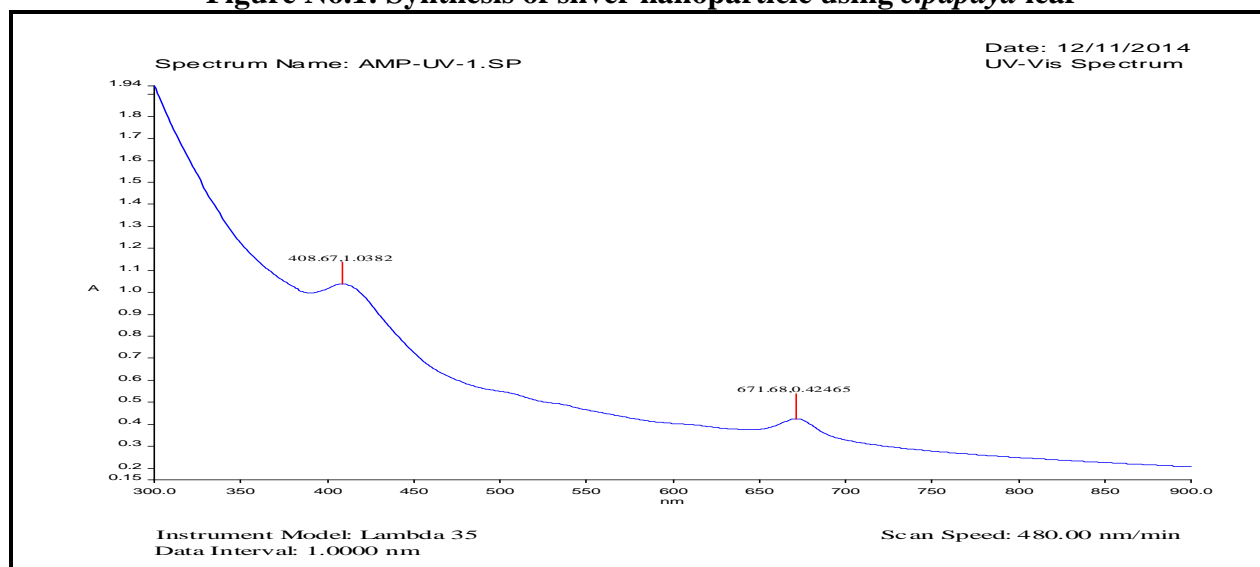


Figure No.2: UltraViolet analysis of synthesized silver nanoparticles

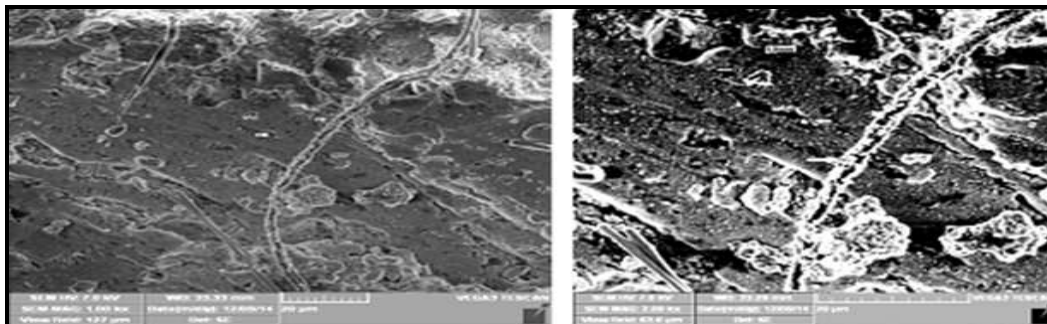


Figure No.3: Scanning Electron Microscopy analysis images showing silver nanoparticles

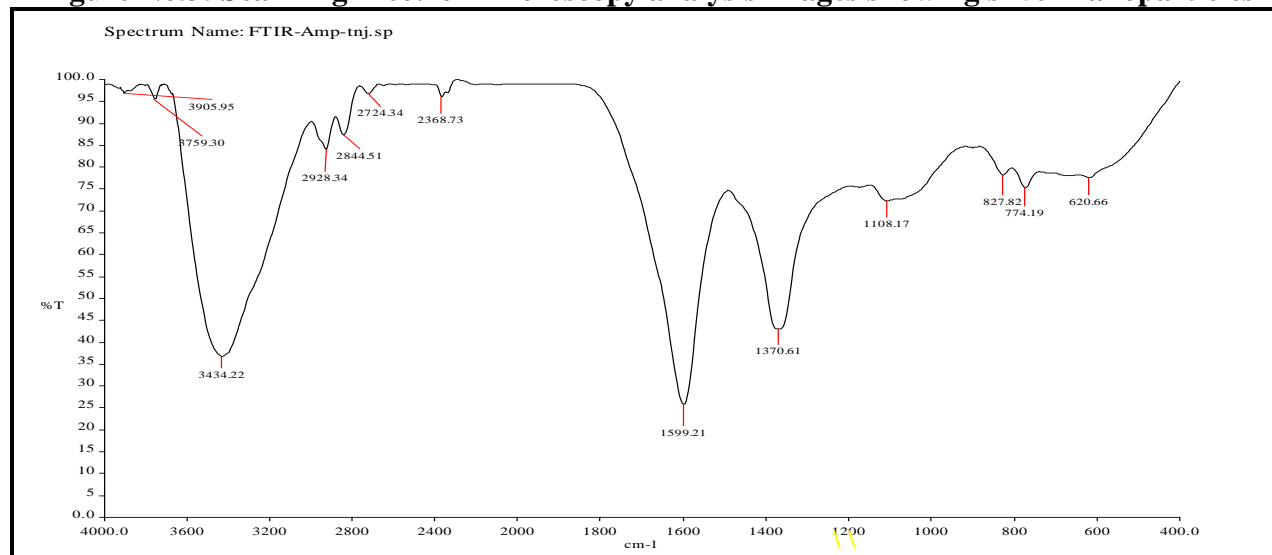


Figure No.4: Fourier transforms infrared spectroscopy analysis of synthesized silver nanoparticles



Figure No.5: Antifungal activity of synthesized silver nanoparticles with *Carica papaya* extract showing zone of inhibition in (mm) in 50 µl

CONCLUSION

To overcome such a problem, Nanotechnology plays a vital role in which, Silver nanoparticles added with *Carica papaya* plant ingredients have potential to control such infections. Active pharma ingredients are natural compounds very safe and small molecules which adheres cell wall naturally while nanoparticle makes pores in membranes and enters with drug molecule, which automatically

traps in cell. Due to action of silver nanoparticle efflux pump can be altered. On the basis of this hypothesis current study were designed and antifungal activity results obtained clearly shows effective action of silver nanoparticle with *Carica papaya* plant extract on three of fungal pathogens. So, such kind of combinatorial therapies should use to manage diseases in future and need more attention to develop more justified antimicrobials.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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