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A Novel Combinatorial Herbal Drug Development Using Nanotechnology against MDR Bacterial Uropathogens

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Abstract: Multidrug resistance in India is direct threat to developing medical technology and sciences. Disease management is somehow tedious task for physicians. Urinary tract infection is one among them; improper diagnosis, unclear symptoms, and resistance due to indiscriminate use of antibiotics are the major reasons. This study designed to develop a combinational therapy of nanotechnology and plant metabolites together for better alternative to traditional medicines. Silver nanoparticles were synthesized using leaves of *Carica papaya.* Synthesized silver nanoparticles were then subjected to check antibacterial activity against clinical isolates i.e. uropathogens like *P. aeruginosa, Klebsiella pneumoniae* and *E.coli* with methanolic plant extract of leaves of *Carica papaya*. Traditional testing were performed with characterization of silver nanoparticles and results shows it can be prove as a promising therapy in future. As compare to traditional antibiotics herbal drug with nanoparticles have more effective bacteriostatic activity.

Key words: Silver nanoparticles, urinary tract infection, antibacterial

INTRODUCTION:

Living organisms are highly adaptable in nature so they are continuous keep on changing as per the condition so called adaptation. Same as micro organisms are also like to be change when environmental changes occur. Due to consistent contact of antibiotics it adapts resistance against sensitive drugs. There may be chances of certain mechanism like efflux pump etc. (1) Since last 20 yrs, *E.coli* is major player of infection and found resistance in sulphonamide and cephalosporin's.(2,3)

Wild diseases like pyelonephritis UTIs, sepsis, etc. serovars are called as uropathogens. There are several bugs who play a crucial role in this kind of soft tissue infection. Infection caused by various routs like invasion through urine canal by soft tissue, or by nephritic ducts. *Klebsiella sp Pseudomonas sp* and *E.coli sp.* are the target and well known terror in this area. Data raised from various epidemiologist reveals 2009, 2011,2013 has severe rise in the resistance strains. (4,5,6,7)

Secretion of multiple-β-lactamases from *Pseudomonas aeruginosa* is a tiresome for current antibiotics due to efflux pump that cause resistance and less effectiveness of antibiotics (8,9) Recently in 2014 August, Gujarat reported highest Amikacin resistance *Pseudomonas aeruginosa* isolated urine sample in India. (10, 11) *K. pneumoniae* have also equal role in the Nosocomial infection spread. Since 1990 it is threat.(12,13)

In last decade herbal drug development has good acceleration in pharma industry due to the side effect and massive use of chemicals in allopathic. Community believes on natural therapy now a days, keeping this in mind several studies reports researched plant metabolites reported in Ayurveda like traditional medicinal systems. By all possible ways scientist trying to get off from this resistance problem. This pilot study also put a hypothesis to hastening the drug development in targeted way. *Carica papaya* plant leaves were included in this study as it known for good antibacterial.(14,15,16) Nanoparticle have same quality to show effect(17,18). Hence, efforts have been made to develop green silver nanoparticle using plant leaves to use against MDR designated uropathogens.

MATERIAL AND METHODS

Preparation of plant extract

Fresh green *Carica papaya* plant leaves were collected. Primarily the leaves were washed with tap water and then with Distilled water. Washed cleaned leaves were dried with dehydration absorbent. Chopped leaves were and dispensed in 100 ml of sterile Deionised distilled water and boiled for one – two hour at 82ºC. 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 BOD bottle separately and to this 100 ml 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 biological activity.

Characterization of Silver nanoparticle:

UV-vis spectra analysis:

The silver nanoparticles were confirmed by measuring Ultra violet radiation absorbance spectra wave length of mixture in the spectrophotometer at a resolution of 1 nm (from 400 600 nm) in normal quartz cuvette within path length

SEM analysis:

The Morphological characterization of the samples was done using SEM analysis. The sample were put in auto fine coater with platinum slide. After that the material was subjected to analysis.

FTIR analysis:

The characterization of functional groups on the surface of AgNPs by plant extracts were investigated by FTIR 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 AgNPs

uniformly in a matrix of dry KBr , compressed to form an almost transparent disc. Pottasium bromide (KBr) was used as a standard analyse the samples.

Screening of Antibacterial activity of plant extract with silver nanoparticle

The modified agar well diffusion method of Perez *et al*.(1990)was employed. Microbial Type Culture Collection (MTCC) were used in this study. *Pseudomonas aeruginosa* (MTCC424) *E.coli* (MTCC443) and *Klebsiella pneumoniae* (MTCC 7028). Each selective medium was inoculated with the microorganism suspended in sterile water. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL/50 µl of the plants extracts and synthesized silver nanoparticles and blanks. The concentration of the nanoparticle employed was 25 µl, and 50 µl., The test was carried out by triplicate. The plates were incubated at 35 ± 2°C for 24 h. The antimicrobial activity was calculated by applying the expression in mm. indicating the zone of inhibition. The antibiotics were used as reference was selected on the basis of survey reported in 2014 in India. Hence only highly sensitive antibiotics applied to concise time.

RESULTS

Biosynthesis of Silver nanoparticles using *C. papaya* leaves

The use of herbal plants as primary health remedies, due to their pharmacological properties, is very common in India, includes flavonoids and phenolics. As per the various literatures have potential to reduce silver nitrate and responsible for biosynthesis of AgNp's. 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, shown in the Fig.1.

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 SPR absorption band. Various literatures have reported the 411nm. We reported on 408nm absorption.Fig.2

SEM analysis:

Scanning Electron Microscopy (SEM) analysis provided the morphology and size details of the nanoparticles. Results shows high density AgNp's synthesized by the plant extract of *C. papaya* more confirmed the presence of AgNp's. The shape of the silver nanoparticles found spherical and hexagonal in some places which look adhered and clumps. Image Fig 3 shows there is thread like structure where which is composed of surface full small dots which are nothing but adhered silver nanoparticles, Size may be ranges from 6nm-12nm.

FTIR analysis : FTIR 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 3905.30,3759cm-1,827cm-1 which are associated OH stretching, C=C stretching, CH stretching, CH stretching respectively. 1599, 1370cm-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 AgNPs from papaya leaves peaks were observed at 3439.72cm-1, and above corresponds to O-H groups, H bonded alcohols and phenols. Discussed in the Fig 4.

Fig 1 Synthesis of silver nanoparticle using *Carica papaya* leaves

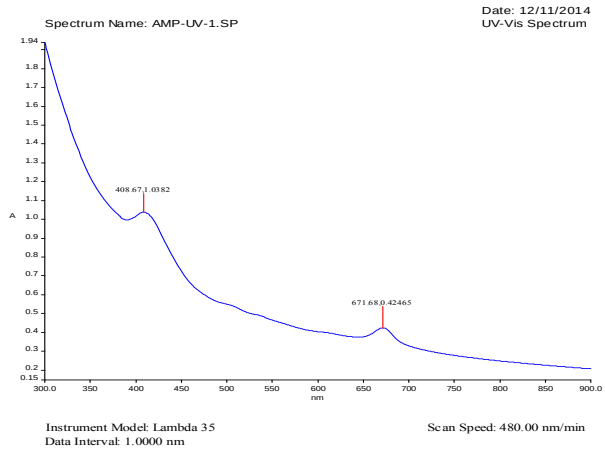


Fig 2. UltraViolet analysis of synthesized silver nanoparticle containing *Carica papaya* plant extract

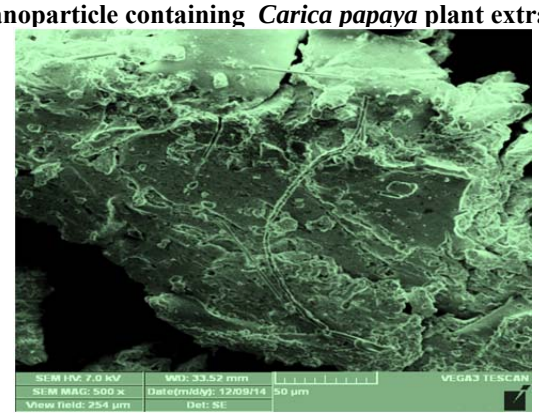


Fig 3 . SEM analysis images showing silver nanoparticles present in *Carica papaya* plant extract.

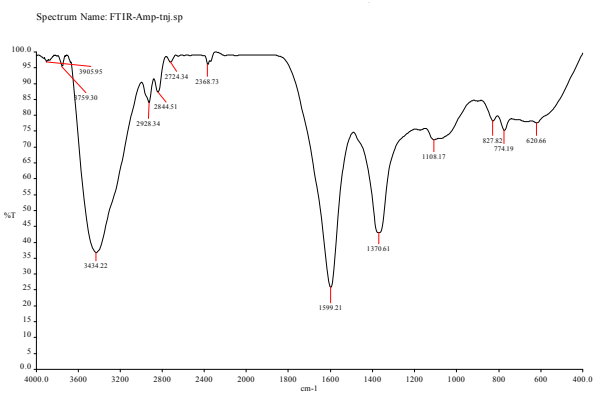
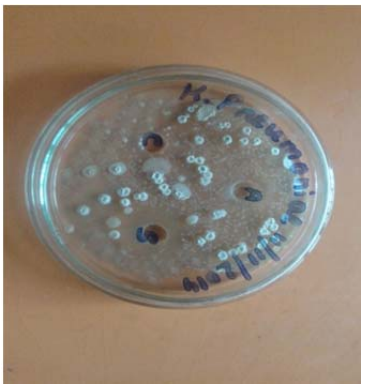


Fig 4 FTIR analysis of *Carica papaya* plant extract showing absorption bands



Klebsilella pneumoniae

E. coli

P. aeruginosa

Fig 5. Zone of inhibition (mm) showing antibacterial activity of plant extract and silver nanoparticles

Table 1 Zone of inhibition (in mm) of clinical isolates at concentration 50(µl) of herbal nanoparticles extract (extract +AgNp)

Test Clinical isolates	Concentration in 25 µl	Concentration in 50 µl	Standard Antibiotics
E. coli	8 mm	9mm	8mm/cef
Klebsiella pneumoniae	10 mm	12mm	7mm/chor
P. aeruginosa	11 mm	11mm	9mm/ceftri

Standard antibiotics used: Cef: Cefpodoxime, Chlor: Chloramphenicol, Ceftri : Ceftriaxone

DISCUSSION:

Various advanced techniques have been employed now a days to understand mechanism of action regarding the antibiotic resistance in bacterial pathogens. Urinary tract infection is one of the deadly infection in the community since long back. Genomics approaches also tried to evaluate the susceptibility of potential targets in bacterial species.(19) *Klebsiella sp*. Are the most common after pseudomonal infection in Nosocomial infections in India, as per data 17% infections are of Klebsiella oriented in lower tract in human.

Niranjan V. & Malini A. (2014) 76.5 % of *E. coli* isolated reported as MDR and isolated from diabetes, chronic renal disease and catherization. (20) The major reason **of** resistant UTIs is the introduction and clonal expansion of competitive, resistant *E. coli* strains in the community. Various factors are responsible for the antibiotics resistance in *E.coli, Klebsiella pneumoniae, Pseudomonas aeruginosa. Klebsiella* develops prominent capsules which interuupts the macrophages to work. Fimbri, LPS, Serum resistance and other factors also plays role in virulence in UTI related *Klebsiella sp*.(21,22)

In case of *Pseudomonas sp.* Catheter related infection, mucosal layer damages leads to invasion of pathogens and disrupts natural barrier which causes bacterial colonisation.(23,24) Reflux pumps is the common reason of antibiotic confrontation in bacterial pathogens. Our results shows synthesis of nanoparticle using *C.papaya* leaves were flourishing and when used with methanolic plant extract it develops as promising outcome in comparing with standard antibiotic in market.

CONCLUSION

Metallic silver nanoparticle synthesis using *C.papaya* leaves were hypothesized keeping their antimicrobial values in mind. Both the components i.e. nanoparticle and metabolites of plant has antibacterial well known develop mechanism. Nanoparticles adhere to the cell wall and increase the pore size of cell membrane which ultimately facilitates the plant metabolites to enter in the cell or interrupts the bacterial colonization. Results in the study demonstrate that there is successful green synthesis of silver nanoparticle using plant leaves. Various analysis for nanoparticles and photochemical analysis were performed in previous study of this project. Antimicrobial study also shows promising results as compare to routine antibiotics. This combinatorial therapy can be use to formulate a plant based nano drug in future to eradicate antibiotic resistance. Route of administration and toxicological levels must be

check in future and pilot study should be design by pharmacists to get perfect in this combinational therapy.

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Conflict of Interest: None declared

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