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Research Article

ONE STEP GREEN SYNTHESIS OF PHYTOCHEMICALS MEDIATED GOLD NANOPARTICLES FROM *AEGLE MARMALES* FOR THE PREVENTION OF URINARY CATHETER INFECTION

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ABSTRACT

Objective: Green synthesizes of phytochemicals capped gold nano particles using *Aeglemarmelos* leaf extract. Characterization of the green synthesized *Aeglemarmelos GNPs.*

Results: The UV- visible spectrum of the bioreduced GNP shoed maximum peak at 536 nm and, structural studies by Scanning Electron microscopyshowed the triangular structure of the GNPs. The elemental composition was confirmed by EDAX. The mono-crystalline nature and the of the green synthesized *Aeglemarmelos* GNPs were confirmed by XRD analysis and the Bragg reflections obtained from the gold nanotriangle clearly correspond to the face center crystalline structure of the gold. The FTIR spectroscopic study has confirmed that the carbonyl group of amino acid residue and peptides of proteins of the plant extract has strong ability to bind metal, and most possibly might have formed a layer on the gold nanoparticles. The green synthesized *Aeglemarmelos*GNPs inhibited about 97 – 100% growth of the clinical pathogens like *Staphylococciaureus, Klebsiella pneumonia, Pseudomonas aeruginosa*and *Enterococcus faecalis.*

Conclusion: Our results encourages that the green synthesized *Aeglemarmelos*GNPs can be further used for the prevention of biofilm formation in catheter related infections after testing for its cytotoxicity properties in cell lines or in animal models.

Keywords: Gold nano particles, *Aeglemarmelos*, One step green synthesize, Antimicrobial.

INTRODUCTION

Many advances in the science of nanotechnology in the past decade and the many new present applications with vast technological abilities haveled to the discovery of nanoparticles of varying sizes and shapes. The field of nanotechnology has recently witnessed spectacular advances in the methodology of nanomaterial's fabrication and utilization of their exotic physicochemical and optoelectronic properties[1]. The development of new chemical or physical protocols are very much concern for environmental contamination because of the large amount of hazardous by-products. So, there is very much a necessity for "green chemistry" which includes a clean, nontoxic, and environment-friendly method of nanoparticles synthesis[2]. As an alternative to conventional methods, biological methods are considered safe and ecologically sound for the nanomaterial's fabrication[3]. The synthesis of nanoparticles by chemicals has long been used in the field of nanotechnology [4], however, the reproducible preparation of stable particles of definite size in a short time can be obtained using biological catalysts, such as bacteria, fungi and plants [5].

Metals like gold, silver, platinum and zinc have been used for the bio-synthesis of nanoparticles having greater potentials than their counter parts,[6] wherein plant extracts have been used. Gold nanoparticles (GNPs) are very important and most widely used nanoparticles with potential applications like antimicrobial agents, water purifiers, air purifiers[7]. The GNP have been synthesized using natural compounds from plants and microorganisms to reduce gold ions to metallic nanoparticles[8].

The synthesis of nano particles using various parts of the plants such as leaf [9], tuber [10], bark [11] and buds[12] are gaining more interest. The methods involvethe use of plant phytochemicals such as terpenoids[13], flavonoids [14], phenol derivatives[15], plant enzymes (hydrogenases, reductases, quinones) and their derivatives, di-hydric phenols [16] as reductants. The mechanism of formation of nano particles by different plant compounds range from proteins to phytophenols[17]. It has been reported that the formation of nano particles by polyols is because of the oxidation of water-soluble compounds to their intermediate products with apparent hydrogen participation of their hydroxyl groups. The particle size has been controlled in situ by some polyphenols present in the plant extracts thatact as natural capping agents. Satyavani et al.,2011 has reported the formation of small sized polyphenol capped nanoparticles by leaf extract of *Citrulluscolocynthis*[18]. The synthesis of nanoparticles using plant extracts is more advantageous over microbial route such as simple and user-friendly process, economical and less reaction time etc[19].

Recent advances in the field of nanotechnology have made it possible to detect microorganisms using nanoparticles functionalized with oligonucleotides complementary to the gene tags of the microorganisms. GNPs were used to detect *Salmonella enteritidis*and *Listeria monocytogenes*, where GNPs deposited within the flagella and in the biofilm network [20]. Similarly, GNP–Poly(para-phenyleneethynylene) could efficiently identify both Gram positive and negative bacteria based on the differential response by each bacteria[21]. The method was found to be simple, easy and highly sensitive with a detection limit of ~0.01 ng/mL. Recent increase in the extent of antibiotic resistance in various microbial pathogens has made it necessary to design suitable methods for the detection of antibiotic resistant organisms. *Aeglemarmelos* is one of the useful medicinal trees popularly known from pre-historic time for its nutritional, environmental, and commercial importance [22]. The leaves contain broadly alkaloids, phenylpropanoids, terpenoids and other polyphenols which were well recognized for their healing power toward wide variety of bacterial and fungal infections [15,23].

Aegelenine (minor alkaloid), coumarin) are the major phytochemical constituents present in the plant. Our attention has been focused, in particular, on Bael - *Aeglemarmelos*(L.) Corr.) An ancient Indian medicinal plant; which has enormous traditional values against various diseases, and many bioactive compounds has been isolated from this plant, through the research activity using modern scientific approaches and innovative tools. Coumarins, marmelosin, marmesin, imperatorin, marmin, alloimperatorin, methyl ether, xanthotoxol, scopoletin, scoparone, umbelliferone, psoralen and marmelide have been reported [24]. There is as much as 9% tannin in the pulp of wild fruits and less in cultivated crops [25]. The antioxidant phytochemicals such as flavonoids, alkaloids, sterols, tannins, phlobotannins and flavonoid glycosides present in the leaf extract possess free radical scavenging activity. Eugenol and

marmesinin present in the leaves have independently shown their antioxidant activity against oxidative stress [26]. In one of our previous studies we have reported the wound healing and Antigenotoxic activities of *Aeglemarmelos* with relation to its antioxidant properties; from our results it is observed that the plant has the wound healing and the anti-genotoxic properties[27].

Various extracts of *Aeglemarmelos* leaves, roots and fruits have been reported to be active against many bacterial strains like *Escherichia coli, Aeromonas sp*., *Pseudomonas salanacearum* and *Xanthomonasvesicatoria*[27]. The ethanolic extract of the root has shown activity against *Vibrio cholerae, Salmonellatyphimurium*, *Klebsiellapneumoniae*, *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*[28]. The ethyl acetate extracts of the plant has exhibited activity against *Vibrio cholerae*, *S. typhi*, *S. aureus, Pseudomonas putida* and *Bacillus anthracis*[29]. Methanol and aqueous extract of *Aeglemarmelos* fruit have shown strong activity against multi drug resistant *S. typhimurium*. The unsaponifiable matter of the seed has shown considerable in - vitro activity against *E. coli*, *S. typhi, Salmonella paratyphi*, *Proteus vulgaris*, Streptococcos *faecalis, V. cholerae*, *Klebsiellapneumoniae*, *P. aeruginosa*and *Neisseria gonorrhoeae*. Both the oil and unsaponifiable matter of the seed have also been found to be active against *B. subtilis, E.coli*, *Klebsiellaaerogenes*, *S. typhi*, *S. paratyphi*, *S. aureus*, *Erwiniacarotovora, Pseudomonas solanacearum, Xanthomonascitri* and *Xanthamasvacearum*[30].

Thus, it is evident that *Aeglemarmelos* has antibacterial activity, and the mechanism of action may be the blockage of protein synthesis either at the transcription or the translation level and the peptide glycan synthesis at the membrane level. The anti-bacterial activity of the leaf extracts may be due to the presence of cumin aldehyde and eugenol because these compounds have been reported for their activities against various bacterial strains[31]. In our earlier studies also we have reported the green synthesis of the many medicinal plant extracts namely *Saracaasoca*[32], *Memecylonumbellatum*[33], *M. edule*[34], *Indigoferaaspalathoides*[35], *Chrysopogonzizanioides*[36] for the synthesis of the silver and gold nanoparticles.

In this study, we report an inexpensive one-pot green synthesis of GNPs at room temperature, stabilized in situ using *A. marmelos* leaf extract. These green-synthesized SNPs of *A. marmelos* were examined by ultraviolet-visible (UV-vis) spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray analysis (EDAX), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analysis for size and shape. The synthesized and well characterized nanoparticles were tested for its antibacterial activity against the clinical pathogens which form biofilm in the urinary catheters.

MATERIALS AND METHODS

One-stepGreen synthesis of Gold Nano Particles

Chloroauric acid ($HAuCl_4$) from Sigma-Aldrich (St Louis, MO, USA) and the aqueous leaf extract of Aegle*marmelos* were used for the bioreduction synthesis of nanoparticles. Five ml of aqueous leaf extract of *Aeglemarmelos* was added to 10 mL of 1 mM aqueous $HAuCl_4$ solution in 250 mL Erlenmeyer flasks and incubated in a Rotary shaker at 150 rpm in dark. The color change in the colloidal solutions occurred, showing the formation of GNPs.[34]

UV-Vis absorbance spectroscopy analysis

The bioreduction of $HAuCl_4$by theaqueous leaf extract of *Aeglemarmelos* was recorded periodically using a UV-Vis 3000+ double beam spectrophotometer (Lab India, Maharashtra, India). The samples were diluted with 2 mL of deionized water and measured for UV-Vis spectrum at regular time intervals. The deionized water was used as a blank for background correction of all UV-VIS spectra. All samples were loaded into a 1 cm path length quartz cuvette for and the UV-Vis spectrometric readings were scanned from 200 to 800 nm and recorded at a scanning speed of 0.5 nm interval. The UV-VIS spectra were fit with Gaussian curves correcting for a cubic background for full-width at half maximum (FWHM) and wavelength of maximum absorbance measurements. The Gaussian fits to the UV-VIS spectra all had goodness of fit values ($\chi^2 \sim 1$), showing accurate curve analysis.[37]

SEM analysis of GNPs

The prepared *Aeglemarmelos* GNPs were characterized using high resolution SEM analysis (JSM-5600LV; JEOL, Tokyo, Japan). The samples were prepared by a simple drop coating of suspended gold solution on to an electric clean glass and allowing the solvent (water) to evaporate. The samples were left to dry at room temperature.

FTIR spectroscopy analysis of *Aeglemarmelos* GNPs after bioreduction

The free biomass residue of the aqueous extract or compound that is not the capping ligand of the nanoparticles are removed by centrifugation at 5000 rpm for 10 minand the resulting suspension was redispersed in 10 mL sterile distilled water. Thereafter, the purified suspension was freeze dried to obtain dried powder[38]. To identify the biomolecules present in the leaf extract of *Aeglemarmelos* and the phytocompounds capped on the GNPs after synthesis were analyzed by FTIR. (*PerkinElmer RX1;PerkinElmer, Waltham, MA, USA)* The bioreducedchlorauric solutions were centrifuged at 10,000 rpm for 15 minutes, and the pellets were washed three times with 20 mL of deionized water.[39] The resulting purified suspensions were dried and ground with KBr pellets and the FTIR spectrum was recorded in the range of 400–4000 cm^{-1}. to obtain a good signal and noise ratio, 512 scans were recorded.[40]

EDAX spectrum measurements

EDAX analysis was carried out for the detection and confirmation of elemental gold. The samples were dried at room temperature and analyzed for the elemental composition of green synthesized *Aeglemarmelos* GNP. The dried *Aeglemarmelos GNP* were drop coated on to carbon film, and tested using EDAX analysis (S-3400N; Hitachi, Tokyo, Japan).

XRD Analysis of green synthesized *Aeglemarmelos* GNPs

The purified *Aeglemarmelos* GNPs were characterized by XRD measurements using an XRD-6000 X-ray diffractometer (Shimadzu, Kyoto, Japan) operated at a voltage of 40 kV and 30 mA with Cu Kα radiation in θ–2θ configurations. The crystallite domain size was calculated from the width of the XRD peaks by assuming that they were free from non-uniform strains using the following Scherer formula,[41]

$$\boldsymbol{D} = \frac{\boldsymbol{0.94\,\lambda}}{\boldsymbol{\beta cos\theta}} (1)$$

Where, D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the FWHM, and θ is the diffraction angle expel the added instrumental broadening [42], the FWHM was corrected using the FWHM from a large-grained Si sample.

$$\boldsymbol{\beta\ corrected} = \left(\boldsymbol{FWHM^2_{sample} - FWHM^2_{si}}\right)^{1/2} (2)$$

This modified formula is valid when the crystallite size is smaller than 100 nm.[43]

Bactericidal activity of *Aeglemarmelos* GNPs

The antimicrobial activity of the *Aeglemarmelos* GNPs was studied against different gram negative and gram positive organisms which form biofilm in the urinary catheters including *coagulase-negative Staphylococciaureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and Enterococcus*faecalis*. The agar dilution method was used for the bactericidal activity of green synthesized *Aeglemarmelos*GNPs in Mueller Hinton Agar which was supplemented with concentrations of2 and 5 µg/ml of green synthesized *Aeglemarmelos* GNPs and each plate was inoculated with 0.1 ml of 10^4CFU /ml of the selected bacteria by spread plate technique. The plates without nanoparticles were considered as control. The number of surviving bacteria in the agar plates was counted after 24 h and 48 h of incubation at 37° C and the percentage of inhibition were calculated to determine the

antibacterial efficiency of the green synthesised phytochemicals capped *Aeglemarmelos* GNPs.

RESULTS

Ultraviolet-Visible spectrometry of the green synthesized *Aeglemarmelos* GNPs

The mixture of the aqueous leaf extract of *Aeglemarmelos* and $HAuCl_4$ solution were subjected to UV–Vis spectroscopy analysis during the rapid bio -reduction process to understand the mechanism of *Aeglemarmelos* GNPsformation. The UV–Visible absorption spectra findings proved a novel technique for preparing the *Aeglemarmelos* GNPs. The Surface Plasmon Resonance (SPR) of the *Aeglemarmelos* GNPsformed corresponded to 536 nm and there was an increase in intensity upto 12 hours as a function of time without any shift in the wavelength peak as shown in Figure-1. From our earlier studies in*M. edule*[34], and*M. umbellatum*[33]and *C. zizanioides*[36], the SPR was observed at 540 nm. Similarly Jayaseelan et al, 2013[44] has reported that aqueous extract of *Abelmoschusesculentus seeds* showed the SPR at 536 nm.

The narrow SPR was observed at lower quantities of the extractbecause of the formation of large anisotropic particles. At lower quantities of the extract, nanoparticle syntheses were not greatly favored because of the absence of sufficient quantity of phytochemicals responsible for capping and efficient stabilization. The fairly sharp SPR band remarked for colloid at 536suggested the formation of triangle shaped *Aeglemarmelos* GNPs.

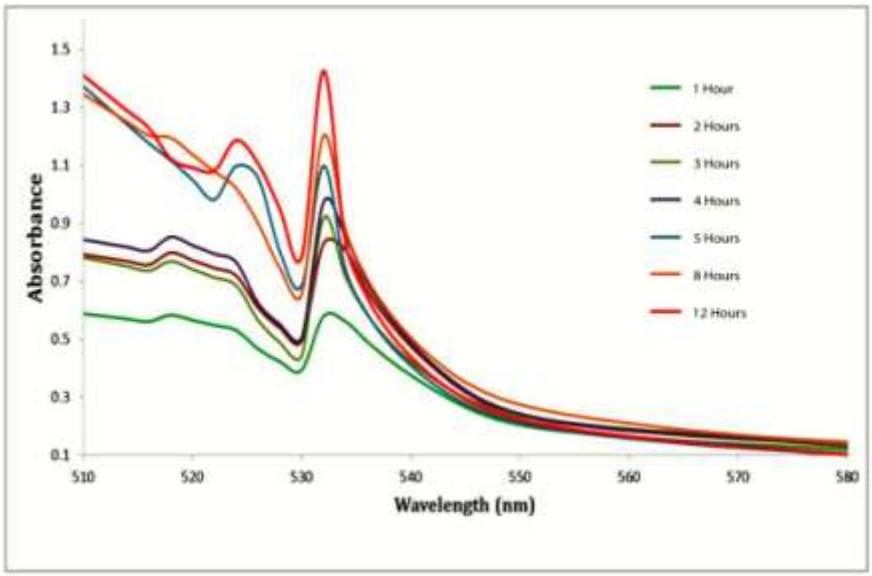

Fig. 1: Time dependent absorption spectra of bioreduced GNP using aqueous extract of *A. marmelos*

Scanning Electron Microscopic Analysis of *Aeglemarmelos* GNPs

A scanning electron microscopic analysis of the *Aeglemarmelos* GNPs were done to determine the structure of the nanoparticles that were formed and shown in Figure 2. From SEM Image it is observed that relatively large populations of flat gold nano triangles were formed. A high magnification SEM image of our previous studies, showed that the biologically green synthesized GNPs using the leaf extracts of *M. edule*[34], *C. zizanioides*[36]and *M. Umbellatum*[33] were predominantly cubic in morphology.

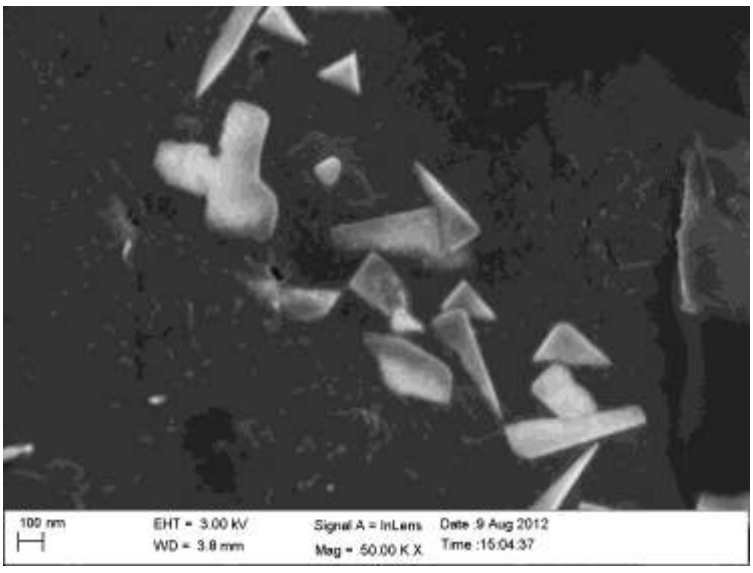

Fig. 2: Scanning electron microscopy image of *Aeglemarmelos* GNPs

EDAX Spectrum of the *Aeglemarmelos* GNPs

The green synthesized Aegle*marmelos* GNPswere further characterized by EDAX analysis, which gave additional evidences for the reduction of chloroauric acid to elemental gold. As seen from the figure 3 the optical adsorption peak was observed at nearly 4.60 keV, which is typical for the adsorption of gold nano crystallites because of SPR. The spectrum showed strong gold signal with weak signals from X-ray emission of the phytochemicls like tannins, flavonoid, saponins and macromolecules such as proteins, aminoacids or enzymes present in the leaf extracts of *Aeglemarmeloes* which were bound to the gold NPs or capped around the gold nanoparticles.

XRD analysis of *Aeglemarmelos* GNPs

XRD analysis of nanoparticles exhibited several size dependent features leading to an irregular peak position, height and width. XRD was mainly done to study the crystalline nature of the green synthesized *Aeglemarmelos*gold nanoparticles. The monocrystalline nature of the green synthesized *Aeglemarmelos* GNPs were confirmed by XRD analysis and the figure 4 shows the X-ray diffraction pattern obtained from the gold nanotriangles. The Bragg reflections obtained from the gold nanotriangle clearly correspond to the face center crystalline structure of the gold. The XRD pattern displays four identical diffraction peaks corresponding to the [111], [200], [220] and [311] appearing at 2θ = 38.22°, 44.55°, 65.6° and 78.6° of metal gold. As described by Verma et al, 2010[45] a very intense Bragg reflection for the [111] lattice is observed, suggesting the [111] oriented gold nanotrianglesare lying flat [21] on the planar surface, while the reflections correspond to [220] and [311] with lattice spacing of 1.44 and 1.23 A° is specific for the triangular morphology. The crystallite sizes of the green synthesized *Aeglemarmelos*GNPs were estimated by the Scherrer equation as 142 nm. The elemental peaks found in the EDAX study agreed with the XRD results.

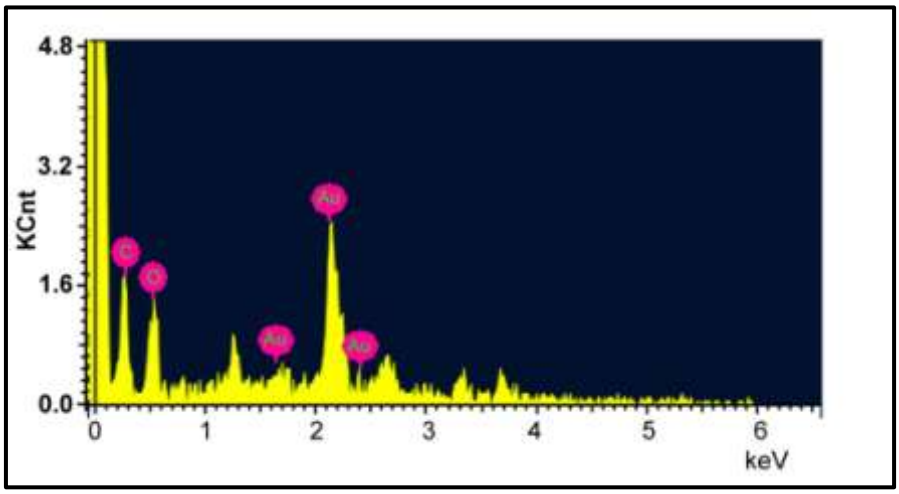

Fig. 3: Energy dispersive X-ray spectrum of *Aeglemarmelos* GNPs.

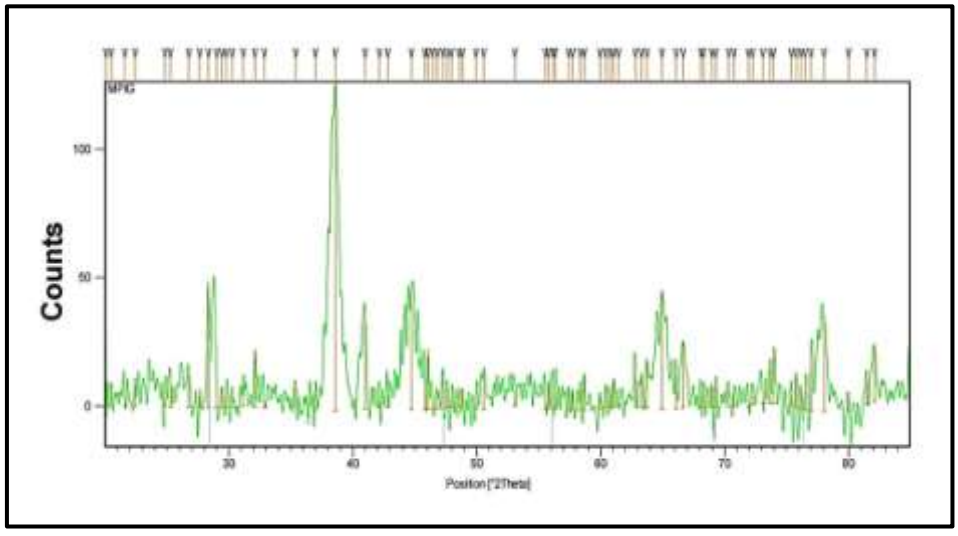

Fig. 4: X-ray diffraction spectrum of green-synthesized *Aeglemarmelos* GNPs.

FTIR Analysis of green synthesized *Aeglemarmelos* GNPs.

The FTIR analysis was performed to identify the possible biomolecules or the phytochemicals present in the *Aeglemarmelos* leaf extract responsible for the reduction and capping on the surface of the gold nanoparticles. The representative FTIR spectra of the green synthesized *Aeglemarmelos* gold nanoparticles are shown in figure-5. The FTIRspectrum of the bio-reduced gold nanoparticles by the phytochemicals adsorption peaks are located at about 1618, 1452, 1378 cm^{-1}. These bands are characteristic of carbonyl stretching vibration in ketones, aldehydes, and carboxylic acids. The characteristic peaks arise because of carbonyl stretch and –N–H stretch vibrations in the amide linkages of the protein.

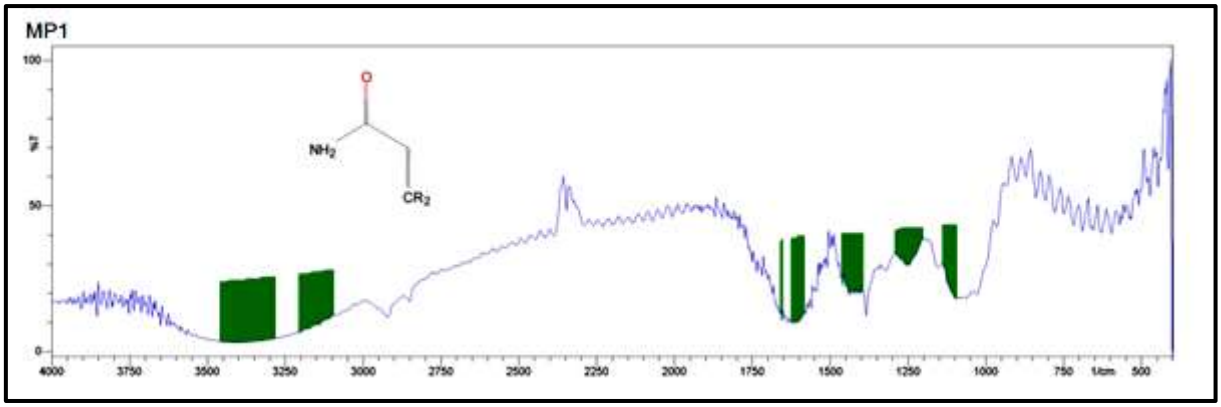

Fig. 5: FTIR Analysis of green synthesized*Aeglemarmelos* GNPs.

The FTIR spectroscopic study has confirmed thatthe carbonyl group of amino acid residue and peptides of proteins of the plant extract has strong ability to bind metal, and most possibly might have formed a layer on the gold nanoparticles. Similar peaks were reported by other researchers that wave numberssignal stretching and vibrational bending these may be derived from compounds such as flavanoids, terpenoids and soluble proteins from plants extracts were responsible for stabilization of gold nano particles. [46]

Antibacterial activity of *Aeglemarmelos* GNP

The antibacterial activity of the green synthesized *Aeglemarmelos* GNPs were tested on the selected clinical pathogens which form biofilm in the urinary catheters. 2µg/ml and 5 µg/ml of green synthesized *Aeglemarmelos* GNPs were used against *Staphylococciaureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and Enterococcus*faecalis* and the percentage of inhibition was calculated from the number of cells survived after 24 and 48 h (figure 6). Compared to the control where no GNPs were added 100% inhibition was observed after 24 and 48 for all the four organisms at 5µg/ml. At 2µg/ml also 100 percentage inhibition was noted for all organisms and after 48 h the percentage inhibition was 96%, 98%, 97% and 100% for *Staphylococci aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Enterococcusfaecalis.*

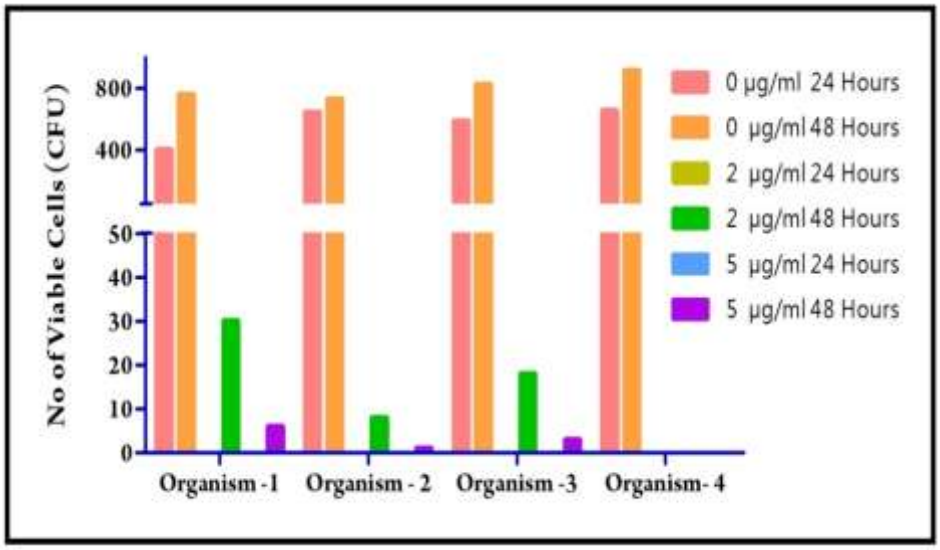

Fig. 6: Antibacterial activities of *Aeglemarmelos*GNPs. Organism 1 - *Staphylococci aureus,* Organism 2 - *Klebsiella pneumonia,* Organism 3 - *Pseudomonas aeruginosa* Organism 4 - *Enterococcusfaecalis*

So it can be concluded that*Aeglemarmelos* phytochemical capped gold nanoparticles can be explored for the prevention of biofilm formation in the urinary catheter related infections after determining the cytotoxicity effect of Aegle*marmelos* GNP. The green synthesized *Aeglemarmelos* GNPs can also be used against the multi-drug resistant pathogens

CONCLUSION

In the present study an effort was made on green synthesis of gold nanoparticles using aqueous extract of *Agelemarmelos* leaves. The water soluble phytocompounds present in the leaf extracts like terpenes, triterpenes, flavonoids, and saponins were mostly responsible for reducing gold ions to nanosized gold particles by capping around the gold particles. The reduction rate of $HAuCl_4$ varied and color intensity changed because of the freaction of the phytocompunds with the gold nanoparticles.The *A. marmelos* induced synthesis of gold nano triangle will provide unprecedented opportunities towards the design and development of engineered 'green' gold Nano trianglesthat can be widely used in many biomedical applications. In conclusion, GNPs can be considered as extraordinary molecular carriers for the targeting,intracellular trafficking and delivery of a huge array of biomolecules including DNA, RNA, proteins, peptides, drugs, genes and other molecules of therapeutic significance. They do not cause significant cytotoxicity

due to their physiochemical properties. Despite these preliminary studies, efforts need tobe taken for designing GNPs to enhance the bioavailability of these functionalized GNPs using the phytochemicals from *A. marmelos* with less immunogenic city and cytotoxicity to be used *in vivo.* A judicious choice between the size and functionalization methodof the GNPs is a prerequisite for the use of GNPs in various biomedical applications[36].

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DISCLOSURE

The authors declare no conflicts of interest in this work.

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