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Phyto-synthesis of gold nanoparticle using waste fruit peel extract and their anti-cancer and anti-microbial activity

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Abstract
Improving environmentally friendly biological procedure for nanoparticles synthesis is one of the key areas of nanotechnology research. Present report focuses on, gold nanoparticles (GNPs) were synthesized at room temperature utilizing *Citrus aurantifolia* (C. aurantifolia) peel extract as reducing agent. The synthesized gold nanoparticles were characterized by UV–Vis spectroscopy, zeta potential (ZP), Scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR). Primarily UV-Visible spectroscopy confirm the GNPs synthesis a SPR at 550 nm. The moderate stability of biosynthesized GNPs were indicated by zeta potential value which was obtainted as -17.80mv. XRD pateern showed that the crystalline nature of GNPs synthesized were of FCC nature. Various biomolecules present in *Citus aurantifolia* peel extract are responsible for stabilization and reduction of GNPs, presence of these biomolecules adentifies by the FTIR analysis. The biosynthesized GNPs fundamentally restrained the development of therapeutically imperative pathogenic gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). It likewise indicated significant anticancerous activity against HepG2 and A549 cell lines. The study recommends that the green synthesized gold nanoparticles could have a high potential for use in the planning of medications utilized against different ailments and were promising contender for some therapeutic applications.

Keywords: *Citrus aurantifolia*, gold nanoparticles, biomolecules, antibacterial activity, anticancerous activity, capping agent, fruit peel extract

Introduction

Nanoparticles, because of their minute sizes and related distinctive properties in correlation with bulkimaterials, give outstanding chance to cross-examine molecular and cellular method with practical clinical applications (De M *et al*., 2008) [6]. Because of their unique features such as catalytic, electrical, optical and magnetic properties, metal nanoparticles have been the focus of much scientific research and these properties have been known to depend considerably on their shape and size. Metal nanoparticles such as Pt, Pd, Au and Ag are commonly used in biological applications, owing to their unique physico-chemical properties (Muthuvel A *et al*., 2014) [19]. Gold nanoprticles (GNPs) are probably the most common element of the metal NPs group due to its possible applications in the field of nanoelectronics, chemical catalysis, surface enhanced Raman scattering, nonlinear optics, disease diagnosis and gene expression. GNPs with biological base are fascinating as they show the finest compatibility with biomolecules (Aromal S A and Philip D., 2012) [2]. For the usual GNPs synthesis, chemical and physical strategies are utilized, for example pyrolysis, chemical reduction, lithography electro-deposition, laser ablation, physical vapor deposition or chemical, sol gel. The greater part of them being exorbitant and requiring the utilization of noxious solvents (Qian W *et al*., 2011, Kumar N *et al*., 2014, Huang H L *et al*., 2012) [23, 15, 19, 12]. On the other hand, these techniques not just utilizing poisonous and costly reagents as stabilizing and reducing agent besides, it is certainly that remaining unreacted harmful chemical substances and by products make GNPs so formed unsatisfactory for use in biomedical applications. To cope up with such tedious techniques and consumption of inconsiderate reducing agents, the attention for this field has moved towards the approach involving green chemistry (Muthuvel A *et al*., 2014) [19]. These days, different natural system, for example, parasites, algae, microbes, yeast and plants are in effect perpetually researched for the synthesis of gold nanoparticles (Zhao P *et al*., 2013, Mittal A K *et al*., 2013) [35, 17]. Among them, plant materials have been specifically noteworthy to scientific society due to their eco friendliness and are profitable over other natural procedures since they eliminate the intricate procedure of maintaining cell structures and can likewise be reasonably scaled up for extensive scale synthesis of nanoparticles (Raju D., 2012, Philip D., 2010, Kumar V G *et al*., 2011) [25, 22, 16]. GNPs synthesis utilizing plant extract has just been accounted with various plants. The banana peel extract intervened GNPs have showed competent anti-bacterial activity towards the majority of the tested fungus and bacterial strain (Bankar A *et al*., 2010) [3]. Moreover, the acquired biofunctionalized GNPs indicated effective anticancerous activity against a range of malignant cell lines. The GNPs were synthesized by G. Mangostana peel extract can be used for biomedical applications and other applications where nontoxicity is crucial (Xin Lee K *et al*., 2016) [30]. GNPs obtained from citrus maxima peel exhibited a high catalytic activity in 4- NP degradation and function as an antibacterial agent against gram negative as well as gram positive bacteria (Yuan C G *et al*., 2016) [33]. *Citrus aurantifolia* is one of the members of *Rutaceae* family and universally distributed in the southeast of Asia, especially in the south of the Yangtze River in China. The peel of *C.*

aurantifolia is the primary byproducts during the process of fruit or juice in food industries (Sood S *et al*., 2009) [27], and it was always considered as biomass waste for further treatments. It has been proved that a variety of flavonoids including narirutin, naringin, hesperidin, eriocitrin and neohesperidin were present in the peels and pulps (Xi W *et al*., 2014) [31]. These kinds of compounds are also known to possess antiviral, anticancer, anti-inflammatory, antiallergenic and analgesic activities. Herein, the peel extract of *C. aurantifolia* can potentially be used as reducing and capping agents for the preparation of AuNPs. Although the study about the biosynthesis of AuNPs using *citrus* flesh or pulps has been reported recently (Yu J *et al*., 2016) [34], there is no report to synthesise noble metal nanoparticles using *C. aurantifolia* peels (Wei Y *et al*., 2016) [29]. Compared with fruit flesh, *C.aurantifolia* peels will be more cost effective. In addition, the growth of plants is usually influenced by regions and climates. How to logically obtain and economically store the biomass is another critical issue for the real applications and scaling-up of phytobiosynthesis methods. *C.aurantifolia* peels can be easily obtained, dried and stored for a long time. These features of *C. aurantifolia* peels enable the proposed method more feasible and practical. To synthesise AuNPs using *C. aurantifolia* peel not only provides us a facile biosynthesis method but also be valuable for the beneficial utilisation of agriculture waste (Yuan C G *et al*., 2016) [33]. AuNPs has different pharmaceutical applications like they were found to be effective against malignant cells (Dipankar C and Murugan S., 2012) [8]. Despite of huge potential of citrus aurantifolia, its medicinal and pharmaceutical applications this work extends the frontiers of c. aurantifolia to the domain of its peel and nanobiotechnology conjointly. As nanoparticle have diverse applications in different aspects of human endeavours with optical, catalytic, pharmaceutical, and biomedical utilizes (Edison T J I and Sethuraman M G., 2012) [9]. Herein, we explain an eco-friendly approach for synthesizing and stabilising GNPs dependent on the reduction of aqueous $AuCl_4^-$ ions by utilizing *C.aurantifolia* peel extract. Likewise, as an organic use of this work, the assessment for antimicrobial and anticancerous activities of bio synthesized GNPs with *citrus aurantifolia* peel extract has been conducted and reported as well. The bio synthesized GNPs were characterized by UV–Vis spectroscopy, zeta potential, Fourier change infrared (FT-IR), spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction.

Materials and Methods

Chemicals and materials

Citrus aurantifolia fresh peels were collected from nearby local market of Bundelkhand University, Jhansi (U.P) India. Chloroauric acid, streptomycin, nystatin, Dulbecco's modified eagles media were procured from Hi-media Pvt. Ltd. Lyophilized cultures of microbial strains and HepG2 and A549 cell lines from the NCCS Pune, India. All other chemicals and solvents used were of analytical grade.

Preparation of aqueous peel extract

Healthy and mature peel of *citrus aurantifolia* were collected and washed thoroughly with triple distilled water to remove dust particles. Fruit peel were cut into small pieces and 25 gm of it was taken with 150 ml double distilled water in soxhlet apparatus. Then it is boiled for 30 min in soxhlet and left at room temperature. The extract was filtered through whatmann filter paper No.1 and used as such for gold nanoparticles synthesis. The extract was freshly prepared for the biosynthesis of GNPs.

Phytochemical analysis

Freshly prepared extracts of peel of *citrus aurantifolia* were qualitatively tested for presence of various phytoconstituents like glycosides, flavanoids, alkaloids, saponins, vitamin, tannins, carbohydrate, amino acid, steroid (Khandelwa K R., 2005) [14].

Synthesis of gold nanoparticles and its conjugates

The aqueous solution of 1mM concentration Chloroauric acid was prepared to synthesize gold nanoparticles from *Citrus aurantifolia* fruit peel extract. 10ml of *Citrus aurantifolia* fruit peel aqueous extract was slowly added to 40ml of aqueous solution of 1mM Chloroauric acid while continous stirring, for reduction into Au ions. The colour change of the reaction mixture from yellow to reddish to dark purple was monitored after 3hr of incubation at room temperature. GNP conjugates are prepared according to one pot synthesis method (Demurtas M and Perry C C., 2014) [7]. GNPs and aqueous solutions of streptomycin and nystatin (15 µg/ml) were prepared and mixed in 1:1 ratio at room temperature. After 12 hr of incubation conjugates are centrifuged, and unreacted chemicals are removed. After centrifugation, the samples were freeze-dried for further analysis using a Eyela FDU-1110 freeze dryer.

Characterization of nanoparticles

The shape, size and stability of the biosynthesized GNPs were characterized using the following techniques.

UV-VIS spectral analysis

For the primary determination of GNPs, UV visible spectroscopy (Thermo scientific varioskan flash multimode microplate reader); at a resolution of 1nm was performed to determine the SPR for the wave length ranging from 300-700 nm.

Scanning Electron microscopy

For this, lyophilized gold nanoparticles are coated on glass slides and observed at 30KV under scanning electron microscope (SEM, JEOL).

Zeta potential measurement

To determine the stability of GNPs a surface charge of biosynthesized GNPs was analyzed using a zeta potential analyzer (Litesizer^{TM} 500 Anton Parr). The estimation of zeta potential depends on the velocity and direction of particles under the influence of known electric field.

Fourier transforms infrared spectroscopy (FTIR)

FTIR spectra of the samples were recorded in KBr pellets utilising an FTIR spectrophotometer (JASCO FT-IR -3600) and spectrum was collected at a resolution of $4cm^{-1}$ in wave number region of 400 to $4000cm^{-1}$ to recognize the probable molecules accountable for the reduction of gold ions and to confirm capped biosynthesized nanoparticles.

X-Ray diffraction (XRD)

XRD investigation of GNPs was done to decide the structural portrayal of the nanoparticles by using X-ray

diffractometer (Rigagu, Kyoto, Japan) working with a Cu anode at 40Kv and 30mA in the range of 2θ value among 20° and 100° with a rate of 2°/min. The intensity of the diffracted X- rays was calculated as a function of the diffracted angle 2θ.

Antimicrobial activity

The antimicrobial capability of bio-synthesized GNPs and *C. aurantifolia* were tested against fungus (*Aspergillus niger* and *Candida albicans*), gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative (*Escherichia coli*, *klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria by Kirby-Bauer disc diffusion method (Bauer A W., 1966) [5]. Streptomycin and nystatin was used as positive control. The test plates were incubated at 37 °C for 24 h and 28 °C for 48 h respectively. After the incubation period, the zone of inhibition (in mm diameter) was observed and tabulated. The experiment was performed in triplicate to avoid any experimental error.

***In -vitro* cytotoxicity studies of green *Citrus aurantifolia* GNPs**

5ml trypsinized cells were centrifuged at 500 rpm for 5min. Cells were then resuspended in complete media. Cells were then counted. Cells were seeded in 96- well plates at 1 * 104 cells / well density and incubated overnight. On Second day cells were treated with gold nanoparticles (10, 25, 50, 100, 200 µg/ml) for 24hrs. 20µl of 5mg/ml MTT was added to each well and incubated at 37 °C. Media was removed. 150µl MTT solvent (DMSO) was added (Wang M *et al*., 2015) [28]. The spectrophotometric absorbance of the color purple blue formazan was measured at 570 nm (Thermo scientific varioskan flash) in a multimode microplate reader. The measurement depends on the reduction of soluble yellow tetrazolium salt by metabolically dynamic cells into insoluble violet formazan crystals. The tetrazolium salt can be taken up by just live cells. The protein (succinate dehydrogenase) found in the live cell's mitochondria will transform over intranalized tetrazolium salt to formazan crystals that are purple in colour.

Results and discussion

The consequences of the fundamental phytochemical screening of aqueous extracts of *citrus aurantifolia* uncovered the presence of phenols, triterpenes, flavonoids, alkaloids, sterols and tannins (Table 1). The phenolic and flavonoids mixes showed a wide extent of natural activities like cancer prevention agent and capping properties.

Table 1: Table showing different Phytochemical Constituent

Phytochemical constituent	Types of test	Observation
Carbohydrate	Benedict's test	++
Tannins	Ferric chloride test	++
Saponins	Frothing	++
Glycoside	Sodium nitroprusside test	--
Flavonoids	Shinoda test Alkaline reagent test	+++
Terpenes and Steroids	Salkowski test	--
Alkaloids	Mayer's test Hager's test	--
Proteins and amino acids	Ninhydrin test Biuret Test	++
Reducing sugars	Legal test	++

Zeta potential measurement

Zeta potential qualities uncover data with respect to the surface charge and stability of green synthesized GNPs. It can be seen from (Figure 1. Zeta potential measurements); the standard Zeta potential value of −17.80 mV showed that the capping atoms present on the surface of GNPs consist mainly of negatively charged groups and are also responsible for moderate nanoparticles stability. In the *C.aurantifolia* peel extract, the affluent resource of flavonoids may be responsible for reducing metal ions and effective stabilization of biosynthesized nanoparticles.

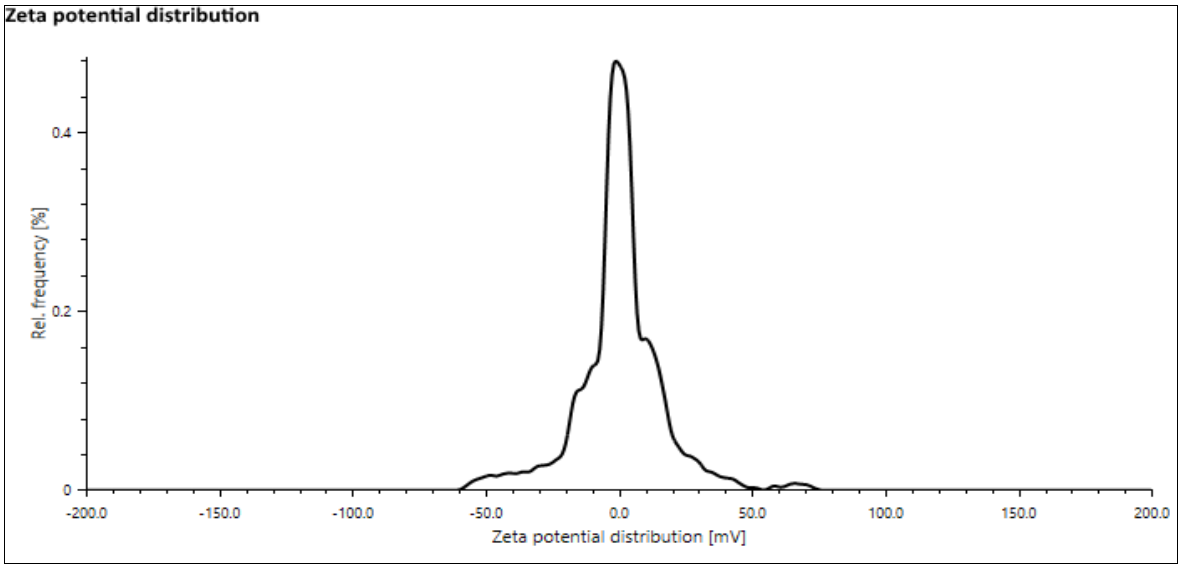

Fig 1: Zeta potential measurements

Nanoparticle composition and Size distribution

The formation of biosynthesized GNPs by chloroauric acid reduction (HAuCl4) with the *Citrus aurantifolia* peel extracts was indicated by a color change in the reaction mixture. It is a preliminarily recognition technique for formation of GNPs (Figure 2). This result also corresponds to gold nanoparticles which was synthesized from leaf extracts of *Catharanthus roseus* (Muthukumar T *et al*., 2016) [18].

Fig 2: Physical appearance of gold nanocolloids

UV-visible spectroscopy confirms the formation and stability of liquid GNPs. The wavelength of UV-Visible was fixed between 300 and 800 nm, the surface plasmon resonance of the GNPs formed corresponds to 550 nm and the intensity increased to 15 min as a function of time without any change in the peak wavelength (Figure 3). This outcome relates to the gold nanoparticles synthesized from leaf extracts of *Carica papaya* (Basavegowda N *et al*., 2014) [4].

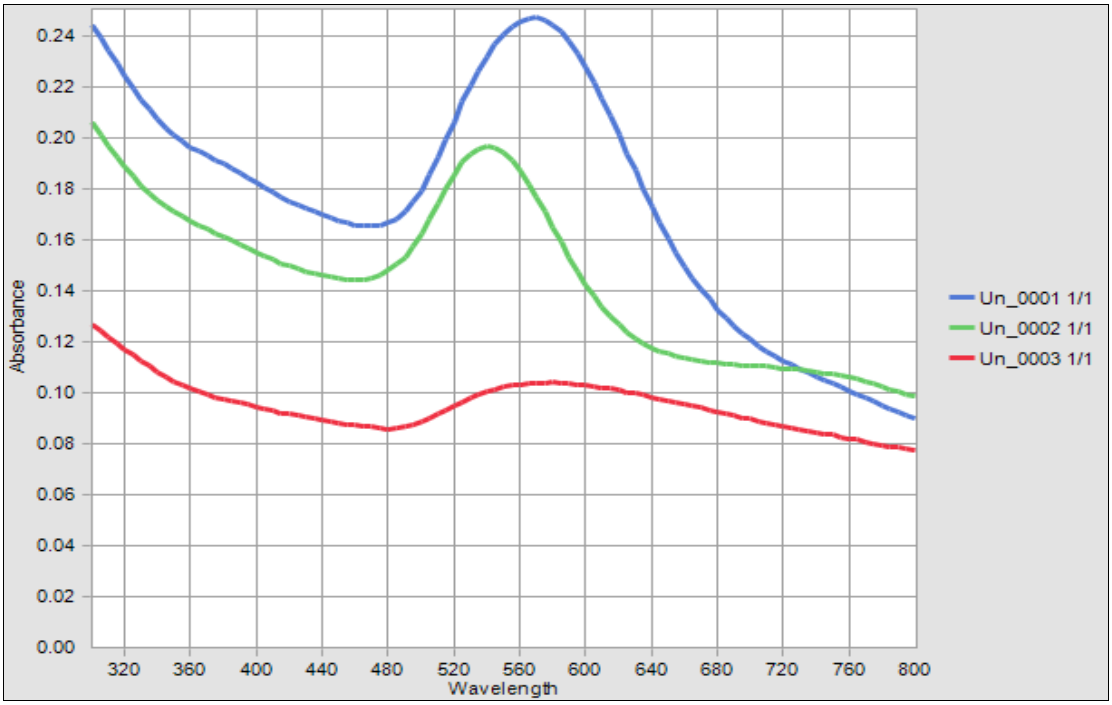

Fig 3: Showing UV –visible absorption spectra analysis

The nanoparticles interface with phytochemical of *Citrus aurantifolia* showed strong peaks at 2921, 2853.2, 1568.8, 1410.2, 1157.0 and 1020.4 cm^{-1} comparative shift in intensity and position allotment were incorrigible with FT-IR, where the intense bands were observed at 2930.3, 1677.0, 1614.5 and 1409.4 cm^{-1}. Contrasting nanoparticles and peel extract FT-IRispectraiit tends to beidistinguished thatithei changesiin the –COOHigroupifori–OH, i.e., hydroxyligroupitheipeakishowed up at 3410.7 cm^{-1}iin crude material, yetiafteriexemplification of nanoparticlesi, theipeakiis narroweriand movedito 3418.5 cm^{-1} and furthermoreifor –C– of carboxylicigroupitheipeak strength diminished afteriencapsulationiofinanoparticlesi. The bandishowingiup at 1387 cm^{-1} compare to C–N stretching of amine group and in the crude extract the peak was wide and blend, however after exemplification of nanoparticlesitheipeakiwasisharper andinarrow. Thisiinfers thatigroup –COOHiin theicompoundiis connected to the GNPs and thereiin aireasonableichange in the spectrai. 1614.5 cm^{-1}iin –C– bondiextending afterithe exemplification thisistretchingiis vanished or masked, similar type of FTIR spectra also seen in gold nanopaticles synthesized from *Citrus sinensis* (Shan M *et al*., 2006) [26].

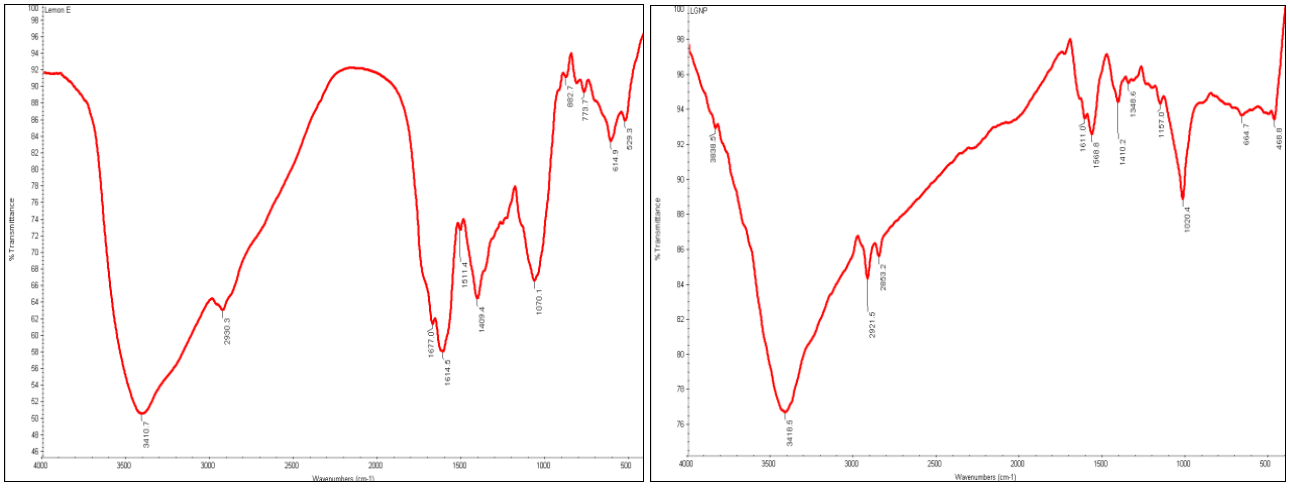

Fig 4: Showing FTIR analysis of (A) Peel Extract (B) Gold Nanoparticles

The SEM images determined the shape and size of the nanoparticles and they are depicted in Figure 5. The particles formed were rod and spherical shape. Theinanoparticlesishaped wereiin theirangeiof 36–50 nmiin sizei. Theinanoparticlesiwere monodisperse, with just a couple of particlesiofivarious sizei.

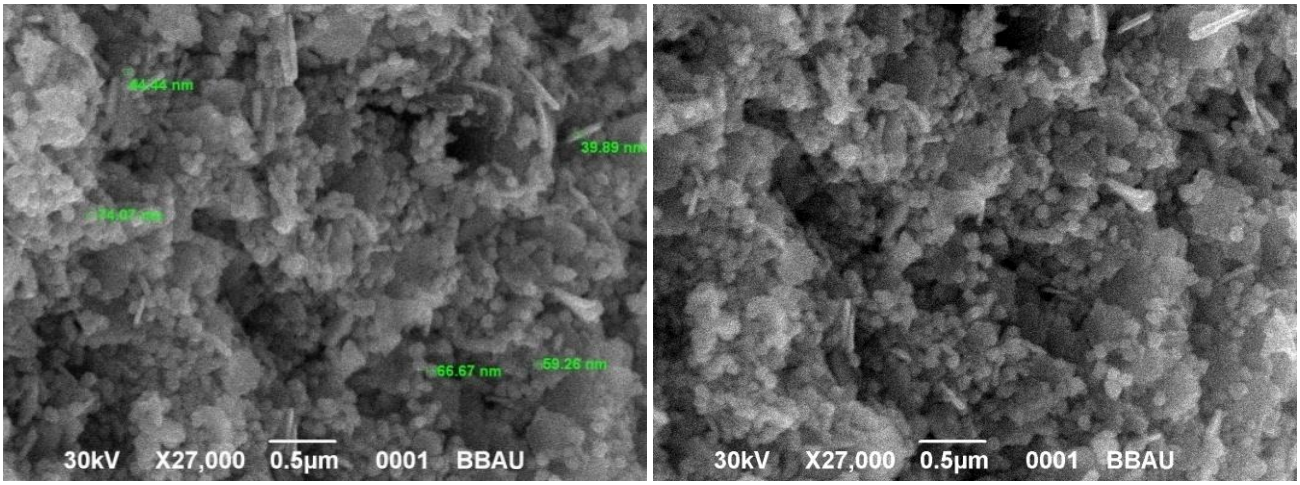

Fig 5: SEM micrographs of the gold nanocolloids

The crystalline idea of GNPs was done utilizing XRD where three diffraction crests were seen in the 2Θ range of 30–80◦, which can be ordered as (111), (200), (220) reflections of face cubic centered structure metallic gold respectively as per Joint Committee on Powder Diffraction Standards record no:04-0784 revealing that biosynthesized GNPs are of unadulterated crystalline gold. The XRD designs (Figure 6) of GNPs got were like the outcomes detailed before. The molecule size of the GNPs obtained were determined utilizing Debye – Scherrer condition which was around 17 nm, were great in concurrence with SEM results too (Yuan C G *et al*., 2016) [33].

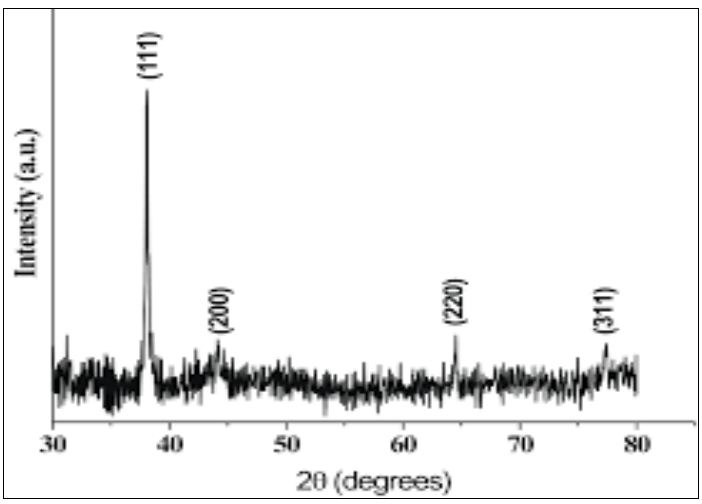

Fig 6: XRD pattern of gold nanoparticles

In EDAX strong signals were seen from the Au atoms in the nanoparticles and weaker signals for carbon and oxygen (Figure 7). The similar type of signals were seen in the GNPs synthesized from *Citrus sinensis* (Shan M *et al*., 2006) [26].

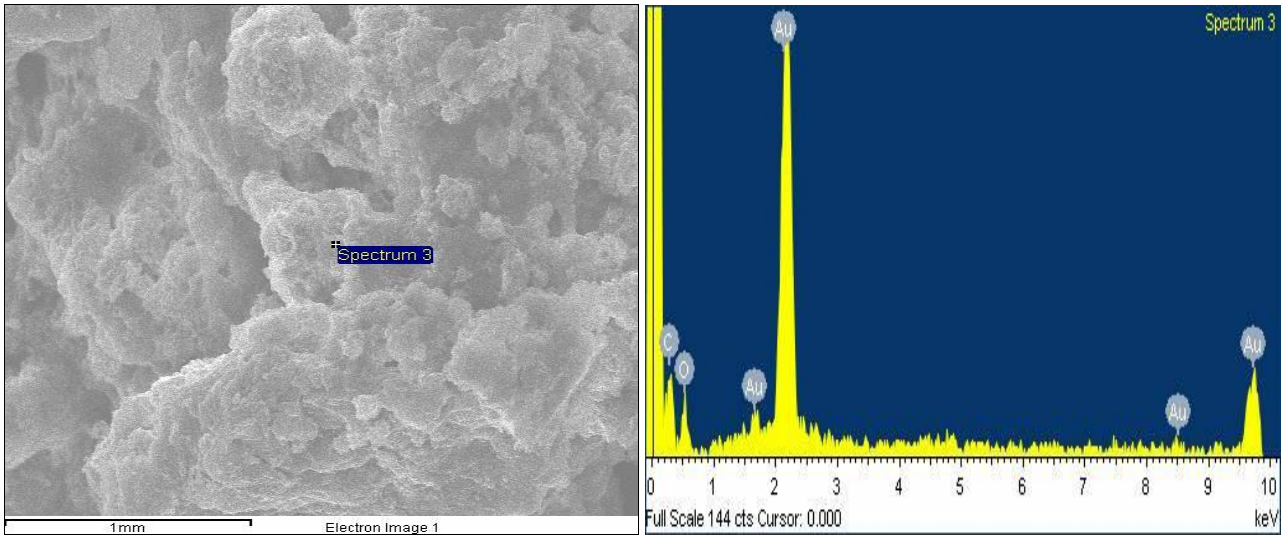

Fig 7: EDX Of Gold Nanoparticles

Antimicrobial and anticancer studies

Antimicrobial properties of Noble metals and their derivatives compounds shows anti-microbial activities that empowers their applications for the healing of burns and interminable injuries. The remarkable natural effectiveness of metal nanoparticles might be credited to the high surface area to volume ratio in contrast with their massive partners (Yallappa S *et al*., 2015) [32]. In this the antibacterial action of biosynthesized GNPs has been inspected on various fungus and bacteria under disc diffusion method (Figure 8) indicates antimicrobial impact of biosynthesized GNPs on various human pathogens *E.coli, K. pneumonia, P.auroginosa, B. subtili, S.aureus, A. niger and C. albicans* correspondingly. The system of the fungicidal and bactericidal impact of metal nanoparticles has not so far been investigated to its full degree. Anyway an obscure description of the method in prior reports refers to a few details for the effect. It has been accounted for that metal nanoparticles may connect to the outer surface of the cell membrane and agitate its control functions for example, respiration and permeability, taking to production of ROS and exhaustion of antioxidants. It is likewise conceivable that metal nanoparticles eject metal particles that enter the cell wall causing protein dysfunctioning and DNA damage (Anand K K H and Mandal B K., 2015, Otari S V *et al*., 2015, Feng Q L *et al*., 2000) [1, 21, 10]. The attachment of the particles to the micro-organisms robustly depends on the surface area accessible for interaction. Consequently smaller GNPs having bigger surface area would give intense bactericidal impact than bigger GNPs. The present investigation plainly demonstrates that the biosynthesized GNPs show great action against Gram negative, Gram positive and fungus. The biosynthesized nanoparticles show significant action against the A549 and HepG2 cell lines at various concentrations. Over 60% of cell death has been experiential for 200 µL concentration of GNPs. The A549 cell line exhibited a decrease in cell viability on increasing the concentration of GNPs (Figure 10). The concentration of antioxidant that exhibits 50% inhibition (IC50) has been observed to be 25µL. The upgraded cytotoxic impact of nanoparticles towards the malignant growth cells is fundamentally because of its simple porousness to the cell boundaries and its solid fondness towards natural macromolecules. It has been recently reported that biogenic GNPs orchestrated utilizing the peel extract of *Citrus aurantifolia* show cytotoxicity movement against HL-60 cells through DNA damage (Geetha R *et al*., 2013) [11]. It has additionally been accounted for that nanoparticles actuate cytotoxicity by means of ROS that's the reason harms to the cell parts through intracellular oxidative stress. The cell viability information obviously shows that the GNPs prompted cytotoxicity is completely based on their shape, size and surface science (Rajan A *et al*., 2015, Nel A *et al*., 2006) [24, 20]. Reports showed the oxidative harm by minute sized GNPs (Jeyaraj M *et al*., 2014) [13]. In this report, A549 and HepG2 cells treated with GNPs show significant anticancer action and are attempted to make a huge imprint in disease treatment.

Fig 8: Antimicrobial activity (A) Anti-bacterial activity (B) Anti-fungal activity

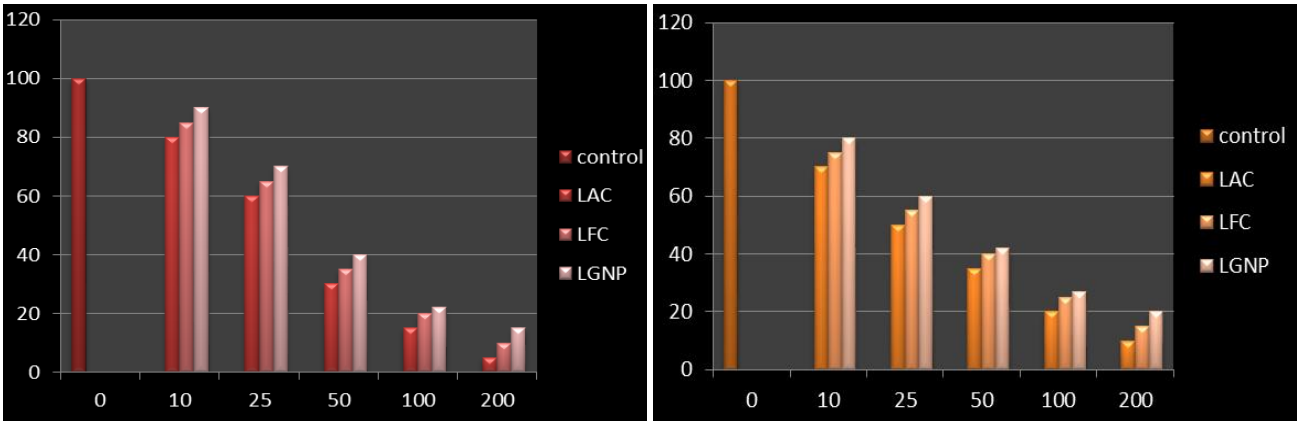

Fig 9: Anti Cancerous activity (A) A549 (B) HepG2

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References

1. Anand KKH, Mandal BK. Activity study of biogenic spherical silver nanoparticles towards microbes and oxidants. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015; 135:639-645.
2. Aromal SA, Philip D. Green synthesis of gold nanoparticles using Trigonella foenum-graecum and its size-dependent catalytic activity. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012; 97:1-5.
3. Bankar A, Joshi B, Kumar AR, Zinjarde S. Banana peel extract mediated synthesis of gold nanoparticles. Colloids and Surfaces B: Biointerfaces. 2010; (80)1:45-50.
4. Basavegowda N, Idhayadhulla A, Lee YR. Phyto-synthesis of gold nanoparticles using fruit extract of *Hovenia dulcis* and their biological activities. Industrial Crops and Products. 2014; 52:745-751.
5. Bauer AW, Kirby WMM, Sherris J, Cand Turck M. Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology. 1966; 45(4)ts:493-496.
6. De M, Ghosh PS, Rotello VM. Applications of nanoparticles in biology. Advanced Materials. 2008; (20)22:4225-4241.
7. Demurtas M, Perry CC. Facile one-pot synthesis of amoxicillin-coated gold nanoparticles and their antimicrobial activity. Gold Bulletin. 2014; (47)1:103-107.
8. Dipankar C, Murugan S. The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts. Colloids and Surfaces B: Biointerfaces. 2012; 98:112-119.
9. Edison TJI, Sethuraman MG. Instant green synthesis of silver nanoparticles using *Terminalia chebula* fruit extract and evaluation of their catalytic activity on reduction of methylene blue. Process Biochemistry. 2012; 47(9):1351-1357.
10. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO *et al*. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. Journal of biomedical materials research. 2000; (52)4:662-668.
11. Geetha R, Ashokkumar T, Tamilselvan S, Govindaraju K, Sadiq M, Singaravelu G *et al*. Green synthesis of gold nanoparticles and their anticancer activity. Cancer nanotechnology. 2013; (4):4:91.
12. Huang HL, Chen JK, Houng MP. Using soft lithography to fabricate gold nanoparticle patterns for bottom-gate field effect transistors. Thin Solid Films. 2012; 524:304-308.
13. Jeyaraj M, Arun R, Sathishkumar G, MubarakAli D, Rajesh M, Sivanandhan G *et al*. An evidence on G2/M arrest, DNA damage and caspase mediated apoptotic effect of biosynthesized gold nanoparticles on human cervical carcinoma cells (HeLa). Materials Research Bulletin. 2014; 52:15-24.
14. Khandelwa KR. "Practical Pharmacognosy" 4th edition, Published by Nirali Prakashan, oct 2005; 183-184:149-156.
15. Kumar N, Komarala V K, Dutta V. In-situ synthesis of Au–CdS plasmonic photocatalyst by continuous spray pyrolysis and its visible light photocatalysis. Chemical Engineering Journal. 2014; 236:66-74.
16. Kumar VG, Gokavarapu SD, Rajeswari A, Dhas TS, Karthick V, Kapadia Z *et al*. Facile green synthesis of gold nanoparticles using leaf extract of antidiabetic potent *Cassia auriculata*. Colloids and Surfaces B: Biointerfaces. 2011; (87)1:159-163.
17. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnology advances. 2013; 31(2):346-356.
18. Muthukumar T, Sambandam B, Aravinthan A, Sastry T P, Kim JH *et al*. Green synthesis of gold nanoparticles and their enhanced synergistic antitumor activity using HepG2 and MCF7 cells and its antibacterial effects. Process Biochemistry. 2016; (51)3:384-391.
19. Muthuvel A, Adavallan K, Balamurugan K, Krishnakumar N. Biosynthesis of gold nanoparticles using Solanum nigrum leaf extract and screening their free radical scavenging and antibacterial properties. Biomedicine & Preventive Nutrition. 2014; (4)2:325-332.
20. Nel A, Xia T, Mädler L, Li N. Toxic potential of materials at the nanolevel. Science. 2006; 311:5761:622-627.
21. Otari SV, Patil RM, Ghosh SJ, Thorat ND, Pawar SH. Intracellular synthesis of silver nanoparticle by Actinobacteria and its antimicrobial activity.

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015; 136:1175-1180.
22. Philip D. Green synthesis of gold and silver nanoparticles using *Hibiscus rosasinensis*. Physica E. 2010; 42:1417-24.
23. Qian W, Murakami M, Ichikawa Y, Che Y. Highly efficient and controllable PEGylation of gold nanoparticles prepared by femtosecond laser ablation in water. The Journal of Physical Chemistry C. 2011; 47(115):23293-23298.
24. Rajan A, Vilas V, Philip D. Studies on catalytic, antioxidant, antibacterial and anticancer activities of biogenic gold nanoparticles. Journal of Molecular Liquids. 2015; 212:331-339.
25. Raju D. Studies on metal accumulation and synthesis of inorganic nanomaterials in plants and their applications, 2012.
26. Shan M, O'Doherty GA. *De novo* asymmetric syntheses of SL0101 and its analogues via a palladium-catalyzed glycosylation. Org. Lett. 2006; 8:5149-5152.
27. Sood S, Arora B, Bansal S, Muthuraman A, Gill NS, Arora R *et al*. Antioxidant, anti-inflammatory and analgesic potential of the *Citrus decumana* L. peel extract. Inflammopharmacology. 2009; 5(17):267-274.
28. Wang M, Yang L, Ji M, Zhao P, Sun P, Bai R *et al*. Aqueous Extract of Terminalia chebula Induces Apoptosis in Lung Cancer Cells Via a Mechanism Involving Mitochondria-mediated Pathways. Brazilian Archives of Biology and Technology. 2015; 2(58):208-215.
29. Wei Y, Fang Z, Zheng L, Tan L, Tsang EP. Green synthesis of Fe nanoparticles using *Citrus maxima* peels aqueous extracts. Materials Letters. 2016; 185:384-386.
30. Xin Lee K, Shameli K, Miyake M, Kuwano N, Bt Ahmad Khairudin N B, Bt Mohamad S E, and Yew Y P. Green synthesis of gold nanoparticles using aqueous extract of Garcinia mangostana fruit peels. Journal of Nanomaterials, 2016.
31. Xi W, Fang B, Zhao Q, Jiao B, Zhou Z. Flavonoid composition and antioxidant activities of Chinese local pummelo (*Citrus grandis* Osbeck.) varieties. Food chemistry. 2014; 161:230-238.
32. Yallappa S, Manjanna J, Dhananjaya BL. Phytosynthesis of stable Au, Ag and Au–Ag alloy nanoparticles using J. sambac leaves extract, and their enhanced antimicrobial activity in presence of organic antimicrobials. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015; 137:236-243.
33. Yuan CG, Huo C, Gui B, Cao WP. Green synthesis of gold nanoparticles using *Citrus maxima* peel extract and their catalytic/antibacterial activities. IET nanobiotechnology. 2016; 5(11):523-530.
34. Yu J, Xu D, Guan HN, Wang C, Huang LK. Facile one-step green synthesis of gold nanoparticles using *Citrus maxima* aqueous extracts and its catalytic activity. Materials Letters. 2016; 166:110-112.
35. Zhao P, Li N, Astruc D. State of the art in gold nanoparticle synthesis. Coordination Chemistry Reviews. 2013; 3-4(257):638-665.