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## Antibacterial Activity of Green Synthesis Gold Nanoparticles from *Lantana camara* Aqueous Extract

Sheela D.<sup>1</sup>, Indra V.<sup>\*1</sup>, Arunagirinadhan N.<sup>2</sup>, Sangeetha B.<sup>1</sup> and Abdul Rahim M.<sup>1</sup>

<sup>1</sup>Department of Zoology, Presidency College, Chennai, Tamil Nadu, India

<sup>2</sup>Central Research Laboratory, Meenakshi Academy of Higher Education and Research (MAHER), Chennai, Tamil Nadu, India

\*Corresponding Author

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**Abstract:** The reduction of aqueous gold metal ions in contact with the aqueous leaves extract of the plant *Lantana camara* results in the formation of gold nanoparticles (AuNPs). Using UV-visible spectroscopy, an absorption peak of gold nanoparticles may be seen at 559 nm. The intensity of the peak (111) at 38.3° diffraction was considerably higher than that of the peaks 200 and 220 at 44.3° and 45.4° planes, respectively, indicating that AuNPs were successfully synthesised. HR-TEM is used to measure mostly spherical particles with sizes ranging from 5 to 50 nm. The FTIR data show that the peaks observed are closely linked to polyphenols, indicating that they may operate as a reducing agent. The antibacterial activity of AuNPs showed the maximum and minimum zone of inhibition. *Staphylococcus aureus* had a greater zone of inhibition in 40 mg/ml, while *Enterococcus* had lower zone of inhibition in ATCC culture while clinical strains of *Staphylococcus aureus* alone had a greater zone of inhibition when compared to other species. Based on the results, it was concluded that AuNPs could be used to combat antibiotic drug resistance.

**Keywords:** AuNPs, *Lantana camara*, HR-TEM, FT-IR, UV-Viz, Phytochemical, Antibacterial activity

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### Introduction

Nanotechnology is the study of nanoscale materials with dimensions ranging from 1 to 100 nm and their applications (Currall *et al.*, 2006). Noble metal nanoparticles have received a lot of interest because of their unique catalytic, electrical, and optical characteristics (Joshi *et al.*, 2008). Because of its distinctiveness, gold nanoparticles (AuNPs) have received a great deal of attention, particularly in biomedication (Baruah

and Dutta, 2009). The diverse surface functionality of AuNPs facilitates nanobiological attachment of AuNPs to drugs (DeRosa *et al.*, 2010, Rakonjac *et al.*, 2011), oligonucleotides (Scheunert *et al.*, 2016, Wiesner and Bottero, 2017), antibodies (Mitter and Hussey, 2019, Bayda *et al.*, 2020), and protein (Rana *et al.*, 2020). Due to their optical properties, Au-NPs can also be used as a marking agent in bioimaging (Mitter and Hussey, 2019). Despite the

popularity and development of chemical and physical methods for manufacturing nanoparticles, there is need to design environmentally acceptable procedures that do not entail the use of hazardous compounds particularly for medicinal purposes (Bayda *et al.*, 2020). To decrease the risks to the environment and humans, synthesis methods that employ natural products as reducing agents require further attention. Greener substrates, such as enzyme (Rana *et al.*, 2020), fungus (El-Sayed and Kamel, 2020), and algae (Aleixandre-Tudo *et al.*, 2020, Svendsen *et al.*, 2020), have been reported to successfully produce Gold nanoparticle. However, in comparison to the challenges encountered in microbe-assisted synthesis (Lateef *et al.*, 2021), plant-mediated synthesis is gaining attraction due to the simplicity with which nanoparticles may be handled and controlled in size and form. Plant-based synthesis is reasonably quick and safe and it may be performed in a room environment without the need for significant physical requirements (Gomez-Marquez and Hamad-Schifferli, 2021). Every part of the plant has been shown to be beneficial, particularly the leaves (Guo and Cui, 2021; Macedo *et al.*, 2021; Park *et al.*, 2021; Salamanca-Buentello and Daar, 2021)

Although few studies have focused on herbal plants (Gottardo *et al.*, 2021). Plants have provided efficient chemotherapeutic treatments for a variety of infectious illnesses, and there is a rising interest in the creation of plant-derived medicines. Many cultures in East Africa, and particularly Uganda, have long used plants to cure a variety of illnesses, including respiratory tract infections. Some believe that they can genuinely treat TB (Ogendo *et al.*, 2003). *Lantana camara* is one of the plants that has been claimed to treat tuberculosis and is commonly utilised in regions of South-western Uganda. *L. camara* is a shrub that belongs to the Verbanaceae family. It is thought to have originated in the Americas' tropics, but it is now found in many African nations, including some dry places, and is common

in Kenya, Uganda, and Tanzania after being introduced as an ornamental plant around 300 years ago (Ogendo *et al.*, 2003; Pereira *et al.*, 2003; Qamar *et al.*, 2005; Verma and Verma, 2006; Sonibare and Effiong, 2008). It is very invasive and has taken over a substantial portion of the vegetative cover since it has been introduced. Its expansion endangers other forms of biodiversity.

*L. camara* has been used for a variety of purposes. It is mostly used as a herbal remedy as a source of microbicides, fungicides, nematicides, insecticides, but it has also been recorded to be utilised as a source of firewood, mulch, hedges. (Gooden *et al.*, 2009; Ghosh *et al.*, 2010; Sathish *et al.*, 2011; Bhagwat *et al.*, 2012; Naz and Bano, 2013). The leaves of the plant are chewed with rock salt and the extract is ingested in traditional usage in South-western Uganda, or they are crushed raw or baked, combined with rock salt, and the extract is used orally for cough therapy. Chemical compounds extracted from *L. camara* leaf extracts have been found to have antimicrobial, fungicidal, insecticidal, and nematicidal action (Kumar *et al.*, 2014; Ajitha *et al.*, 2015; Kumar *et al.*, 2015). There are also claims that lantana chemicals extracted from leaf extracts can be used as weed killers and have been tried with some effectiveness against water hyacinth. It is also claimed that verbacoside, a chemical derived from lantana extract, has anti-microbial, immunosuppressive, and anti-tumour properties (Swamy *et al.*, 2015; Tadesse *et al.*, 2017; Bandi *et al.*, 2018).

It has been reported that lantana oil can be used to cure skin itches, as an antiseptic for wounds, and as an external therapy for leprosy and scabies (Aritonang *et al.*, 2019; Liu *et al.*, 2019; Chowdhury *et al.*, 2020; Devi and Khwairakpam, 2020). Lantana extracts are used in folk medicine to treat malignancies, chicken pox, measles, asthma, ulcers, swellings, eczema, tumours, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria, and atoxy of abdominal viscera (Etuh *et al.*, 2021;

Kato-Noguchi and Kurniadie, 2021; Anwar *et al.*, 2021; Delgado-Altamirano *et al.*, 2021). The main task of this study is threefold. For growth inhibition the bacteria chosen is the Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Enterobacter*, *Staphylococcus aureus* and *Enterococcus faecalis*. To the best of our knowledge, no study has been published on the use of the *Lantana camara* in the production of AuNPs or other metal nanoparticles. We show the production and characterisation of AuNPs using Gold (III) chloride hydrate and aqueous *Lantana camara* leaves extract and also we have identified the antibacterial potential of the green synthesized nanoparticles against the clinical strains and ATCC strains.

## Materials and Methods

The plant leaves of *Lantana camara* (Fig. 1) were collected from Valasamalai Kundru, Tiruvanamalai District Tamilnadu, India and were identified and authenticated by Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC) West Tambaram Chennai, Tamilnadu, India.



Fig. 1: *L. camara*.

### Qualitative Phytochemical Analysis:

Qualitative Screening of phytochemicals present in the plants *Lantana camara* were analysed according to the methods of Kamal (2014).

#### Test for Acids:

**Millon's Test:** To 1.0 ml extract, five drops Millon's reagent was added, heated on a water bath for 5 min and allowed to cool, followed by addition of

1% sodium nitrite solution. Formation of red colour indicates the presence of acids.

#### Test for Alkaloids:

**Mayer's Test:** To 2.0 ml extract, 2.0 ml concentrated hydrochloric acid followed by few drops Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

#### Test for Anthocyanin and Betacyanin:

**Sodium Hydroxide Test:** To 2.0 ml extract, 1.0 ml 2N sodium hydroxide was added and heated for 5 min at 100 C. Formation of bluish green colour indicates the presence of anthocyanin and yellow colour shows the presence of betacyanin.

#### Test for Carbohydrates:

**Molisch's Test:** To 2.0 ml extract, 1.0 ml Molisch's and few drops of concentrated sulphuric acid were added. Formation of purple or reddish ring indicates the presence of carbohydrates.

#### Test for cardiac glycosides:

**Ferric Chloride Test:** To 0.5 ml extract, 2.0 ml glacial acetic acid and few drops 5% ferric chloride were added. This was under layered with 1.0 ml concentrated sodium hydroxide. Formation of the brown ring at the interface indicates presence of cardiac glycosides.

#### Test for Coumarins:

**Sodium Hydroxide Test:** To 1.0 ml extract, 1.0 ml 10% sodium hydroxide was added. Formation of yellow colour indicates presence of coumarins.

#### Test for Flavonoids:

**Sulphuric Acid Test:** 1.0 ml extract was treated with few drops of concentrated sulphuric acid and observed for the formation of orange colour, which indicates the presence of flavonoids.

#### Test for Glycosides:

**Sulphuric Acid Test:** To 2.0 ml extract, 1.0 ml glacial acetic acid, 5% ferric chloride and few

drops concentrated sulphuric acid were added. Presence of greenish blue colour indicates the presence of glycosides.

*Test for Phenols:*

*Ferric Chloride Test:* To 1.0 ml extract, 2.0 ml distilled water, followed by few drops 10% ferric chloride were added. Formation of blue or green colour indicates presence of phenols.

*Test for Proteins:*

*Ninhydrin Test:* To 2.0 ml extract, few drops 0.2% ninhydrin was added and heated for 5 min. Formation of blue colour indicates the presence of proteins.

*Test for Quinones:*

*Sulphuric Acid Test:* To 1.0 ml extract, 1.0 ml concentrated sodium hydroxide was added. Formation of red colour indicates the presence of quinones.

*Test for Saponins:*

*Foam Test:* To 1.0 ml extract, 5.0 ml distilled water was added and shaken well in a graduated cylinder for 15 min lengthwise. Formation of 1.0 cm layer of foam indicates the presence of saponins.

*Test for Starch:*

*Iodine Test:* To 2.0 ml extract, few drops iodine solution was added. Formation of blue-purple colour indicates the formation of starch.

*Test for Steroids:*

*Salkowski Test:* To 5.0 ml extract, 2.0 ml chloroform and few drops concentrated sulphuric acid were added. Formation of red colour indicates the presence of steroids.

*Test for Tannins:*

*Ferric Chloride Test:* To 1.0 ml extract, 2.0 ml 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

*Test for Terpenoids:*

*Sulphuric Acid Test:* To 0.5 ml extract, 2.0 ml chloroform was added and to this, concentrated sodium hydroxide was added carefully. Formation of red brown colour at the interface indicates presence of terpenoids.

*Test for Triterpenoids:*

*Libemann-Buchard Test:* To 1.5 ml extract, few drops Libemann-Buchard reagent (acetic anhydride and concentrated sodium hydroxide) was added. Formation of blue green colour indicates presence of triterpenoids.

*Preparation of Lantana camara leaf extracts using solvents:*

Fresh leaves of *L. camara* were collected and washed thoroughly using running tapwater and then finally washed with double distilled water, shade dried at room temperature to remove moisture. Then the dried leaves were ground into fine powder using hand blender. The powdered leaves were used for Green Synthesis of Gold Nanoparticles.

*Green Synthesis of Gold Nanoparticles:*

To 1g of fine powdered leaves, 10 ml of distilled water were mixed in 50 ml of beaker. The mixture was boiled for 30 min and stirred in a magnetic stirrer at 700 rpm. The aqueous leaves extract obtained were filtered using Whatman No.1 filter paper. To the 5 ml of aqueous leaves extract, 45 ml of 1 mM Gold (III) chloride hydrate aqueous solution was added. Colour change was observed which indicates the reduction of Au<sup>+</sup> ions to Au<sup>0</sup>. The gold nanoparticles formed were centrifuged at 15000 rpm for 30min at 37 C. The supernatant were discarded and then fine sediment pellets were centrifuged again with distilled water to eliminate any biomaterials that could interfere with the construction of the synthesized Gold Nanoparticles (AuNPs).

*Characterization of green synthesized Gold Nanoparticles (AuNPs):*

*UV-Visible spectral analysis:*

The colour change was observed in the Gold (III) chloride hydrate solution incubated with *L. camara* leaf aqueous extract. The bio-reduction of ions Gold (III) chloride hydrate in solution was monitored by periodic sampling of aliquots (0.1 ml) of aqueous component and measuring the UV-Vis spectra of the solution in 10-mm-optical-path-length quartz cuvettes with an UV-2600, SHIMADZU spectrophotometer at a resolution of 1 nm between 300 and 800 nm with a scanning speed of 1856 nm/min. The AuNPs solution was diluted with deionized water to avoid errors due to high optical density of the solution.

#### *Fourier Transform Infra-Red (FT-IR) spectroscopy:*

For FT-IR measurements, the bio-reduced Gold (III) chloride hydrate solution was centrifuged at 10,000 rpm for 15 min and the pellet was washed with deionized water to get rid of the free proteins/enzymes that were not capping the gold nanoparticles. The samples were dried and ground well with KBr pellets and analysed on aIR Tracer-100, SHIMADZU spectrum one instrument in the diffuse transmittance mode operating at a resolution of 4 cm<sup>-1</sup> over 3800–600 cm<sup>-1</sup>. In order to obtain a good signal/noise ratio, 512 scans were recorded.

#### *X-ray diffraction (XRD) studies:*

The bio-reduced Gold (III) chloride hydrate solution was drop-coated onto glass substrate and powder X-ray diffraction measurements were carried out on a PANalytical X'pert PRO X-ray diffractometer. The pattern was recorded by Cu K<sub>1</sub> radiation of 1.5406 Å and nickel monochromatic filtering the wave at tube voltage of 40 kV and tube current of 30 mA. The scanning was done in the region from 30 C to 80 C at 0.02/min and the time constant was 2 sec.

#### *High Resolution Transmission Electron Microscopy (HR-TEM) with SADE:*

Colloidal suspensions of AuNPs were ultrasonicated for 15 min and analysed using JEOL-1200EX TEM (Tecnai™ G2 20 U-Twin) operating at 80 kV.

#### *Antibacterial Activities:*

To observe the antibacterial potential of *Lantanacamara* green synthesized Gold Nanoparticles (AuNPs) against human pathogenic bacteria such as--

Clinical strains - *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, *Efaecalis* and *Enterobacter*.

ATCC *E. coli* (25922), *K. pneumonia* (13883), *P. aeruginosa* (25873), *Staphylococcus aureus* (25923), *E. faecalis* (29212) and *Enterobacter* (200321) culture were used in this study using well diffusion method (Vijilvani *et al.*, 2020).

## **Results and Discussion**

#### *Gold nanoparticle (AuNPs) synthesis using L. camara leaves aqueous extract:*

The *L. camara* leaves aqueous extract used for the reduction of Au<sup>+</sup> ions to Au<sup>0</sup> was prepared by adding 1 g of leaves powder in a 50 ml beaker with 10 ml distilled water. The mixture was then boiled for 30 min in magnetic stirrer with a rpm of 700. In a typical experiment, the obtained leaves aqueous extraction was filtered using Whatman No. 1 filter paper and filtered to get the pure transparent extract. 5 ml of the leaves aqueous extract was added to 1 mM aqueous Gold (III) chloride hydrate solution (0.5 ml). Colour change is due to excitation of Surface Plasmon Resonance (SPR) vibration, which indicated by reduction of Au<sup>+</sup> ions to Au<sup>0</sup> as shown in the Figure 2. After the completion of the reaction, AuNPs were spun at 15,000 rpm, for 30 min at 37 C, the supernatant was discarded and then fine sediment pellet was centrifuged again with distilled water to eliminate any biomaterials that could interfere with binding of Au<sup>+</sup> to the biomass or construction of the AuNPs (Fig. 2).

#### *Possible mechanism of AuNPs synthesis:*

Upon mixing the aqueous leaves extracts of *L. camara* with aqueous Gold (III) chloride hydrate solution, the solution transmuted color rapidly from dark brown to violet, indicating the formation of AuNPs. The formed AuNPs exhibit a

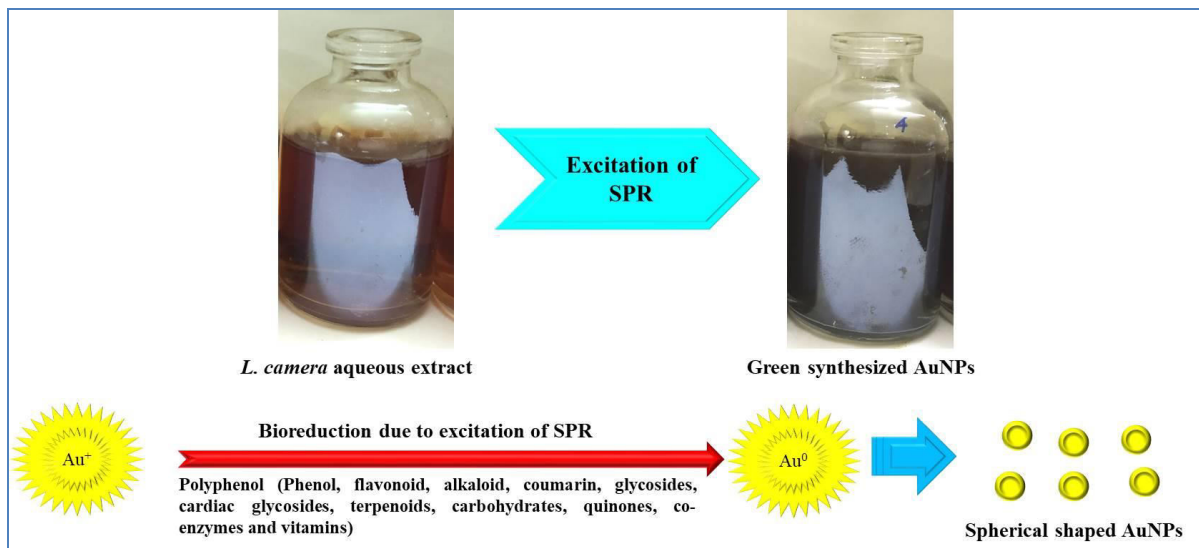


Fig. 2: Bioreduction of AuNPs.

visible violet colour due to the localized SPR. The accepted hypothetical mechanism for the synthesis of nanoparticles in this way is by a phytochemical-driven reaction in which the plant extracts contain complex reducing molecules such as antioxidants, enzymes, and phenolic moieties, which reduce gold cations into AuNPs. The hypothetical reduction of Gold (III) chloride hydrate is driven by the presence of the phytochemicals to form zero valent gold, which will subsequently lead to the agglomeration of gold atoms to nanosized particles, which are finally stabilized by the phytochemicals to give isotropic (spherical) AuNPs.

Photosynthetic plants, including *L. camara*, contain a complex biological network of antioxidant metabolites and enzymes that work collectively to prevent oxidative damage to cellular components such as carbohydrates, polyphenols, flavonoids, terpenoids, triterpenes, alkaloids, tannins and proteins, which could act as reductants for metal cations, leading to the formation of AuNPs. It is also probable that acids, alkaloids, anthocyanins and betacyanins, carbohydrates, cardiac glycosides, coumarins, flavonoids, glycosides, phenols, proteins, quinones, saponins, steroids, tannins, terpenoids and triterpenoids can reduce gold ions to form nanoparticles at an elevated temperatures.

Proteins, phenolics, and other chemical compounds within leaf extracts can reduce gold salts and provide exquisite tenacity toward the agglomeration of the formed nanoparticles. In *L. camara* leaves aqueous extract, all the secondary metabolites play crucial roles in the bio-reduction of metal ions, yielding nanoparticles as shown in Table 1.

#### UV-Visible spectral analysis:

Gold nanoparticles exhibit a distinct optical feature commonly referred to as Localized Surface Plasmon Resonance (LSPR), that is, the collective oscillation of electrons in the conduction band of gold nanoparticles in resonance with a specific wavelength of incident light. LSPR of gold nanoparticles results in a strong absorbance band in the visible region (500 nm - 600 nm), which can be measured by UV-Vis spectroscopy. The LSPR spectrum is dependent both on the size, and shape of gold nanoparticles as shown in the Figure 3. Upon binding of aqueous extracts to the gold nanoparticle surface, the LSPR spectrum will red-shift by a few nanometers, as shown in Figure 3. This shift is a result of an increase in the local refractive index at the gold nanoparticle surface, and is the basis of label-free SPR biosensing. For particles with uneven shapes such as gold nanourchins or gold nanorods this local refractive index change is more profound due to a further

Table 1: Qualitative phytochemical analysis of *L. camara* aqueous extract

S. No.	Phytochemicals	<i>L. camara</i>
1	Acids	++
2	Alkaloids	++
3	Anthocyanins and Betacyanins	+++
4	Carbohydrates	++
5	Cardiac Glycosides	++
6	Coumarins	++
7	Flavonoids	++
8	Glycosides	+++
9	Phenols	+++
10	Proteins	+
11	Quinones	+
12	Saponins	+
13	Steroids	+
14	Tannins	+
15	Terpenoids	++
16	Triterpenoids	++

+ Present and – Absent

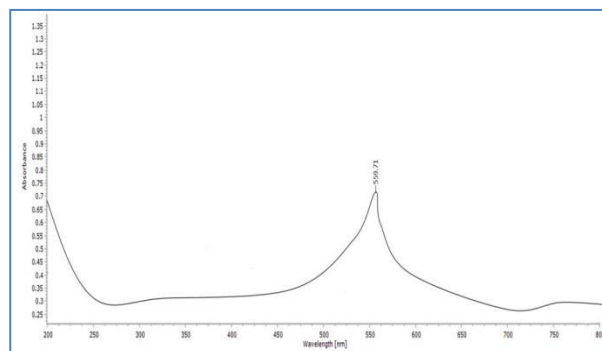


Fig. 3: UV-Vis spectral image of *L. camara* AuNPs.

enhancement of the electromagnetic field at points of "unevenness" at the particle surface. It can be observed that the reduction of gold ions reaches saturation within 10 min of reaction, and after that, only slight variations can be noted in the intensity of SPR bands. This result indicates that the reaction is completed in 10 min. Figure 4 showed that there is no obvious peak for *L. camara* aqueous extract.

XRD patterns of the AuNPs synthesized *L. camara* aqueous extract displayed Bragg reflections representative of the face centred cubic (fcc) structure of gold (Fig. 5). The intensity of the

peak of 111 at 38.3° diffraction was much stronger than those peaks of 200 and 220 at 44.3° and 45.4°, respectively. The mean size of the AuNPs was also calculated using the Debye–Scherrer equation by determining the width of the 111 Bragg reflection. The mean size and shape of the AuNPs determined from XRD patterns confirmed the data analyzed from TEM images. In the case of the optimum aqueous core extract, the average diameter obtained from TEM and XRD analysis was in the range of 5–50 nm, respectively. As the Debye–Scherrer equation is best applicable to highly monodispersed nanoparticles.



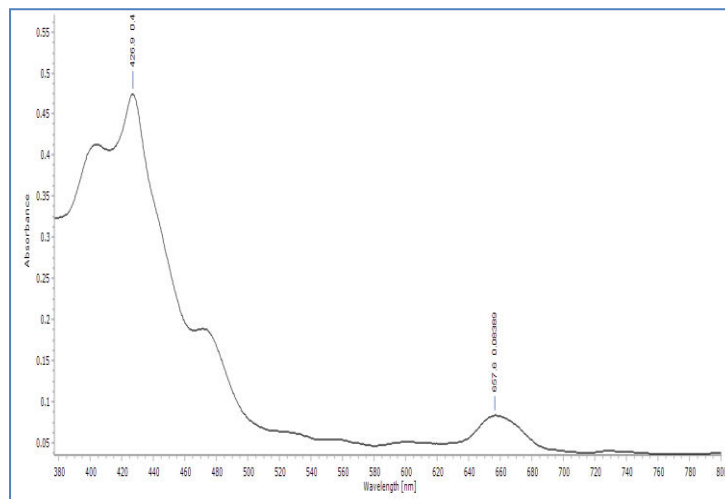


Fig. 4: UV-Vis spectral image of *L. camara* aqueous extract.

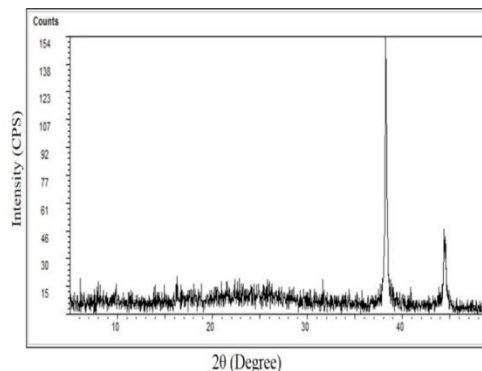


Fig. 5: XRD spectral image of *L. camara* AuNPs.

#### FTIR spectral image:

Figures 6 and 7 show the FTIR spectra of *L. camara* aqueous extract and AuNPs. *L. camara* leaves aqueous extract revealed a number of intense bands at  $915.3\text{ cm}^{-1}$  (C-F),  $1182\text{ cm}^{-1}$  (C-F),  $1294\text{ cm}^{-1}$  (C-O-C stretch),  $1492\text{ cm}^{-1}$  (C=C aromatic) and  $2999\text{ cm}^{-1}$  (-C-H stretch). The FTIR spectrum of AuNPs bio-reduction showed the bands at  $1492\text{ cm}^{-1}$  (C=C aromatic) and  $3006\text{ cm}^{-1}$  (=C-H stretch).

#### HR-TEM analysis:

HRTEM image of AuNPs (Fig. 8) showed mostly the presence of spherical or ovoid nanoparticles in the range of 5-50 nm. As observed in the figures, the AuNPs showed ovoid structure with little

aggregation. This size was also in accordance with XRD analysis. Several TEM images are presented in Figure 8 as examples of the AuNPs shapes formed when the plant extracts were used. In some cases, these shapes corresponded to single crystals, as can be deduced from their diffraction patterns. Since the HR-TEM images show only a few particles in each picture, statistically reliable distributions of these shapes and sizes cannot be evaluated.

According to the results of the two investigations, gold nanoparticles stabilised with matured leaves provide a more stable absorption peak than aqueous extract. In comparison to the citrate stabilised gold nanoparticle, matured leaves provide a similar UV-Vis spectrum,

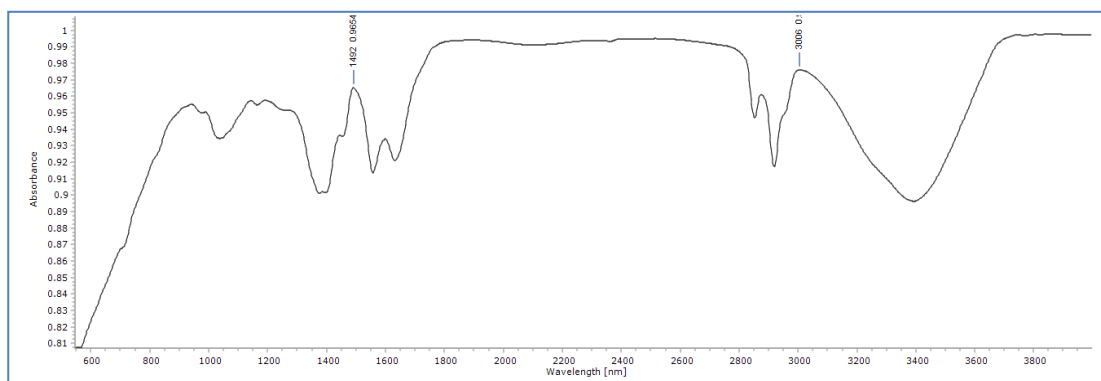


Fig. 6: FTIR spectral image of *L. camara* aqueous extract.

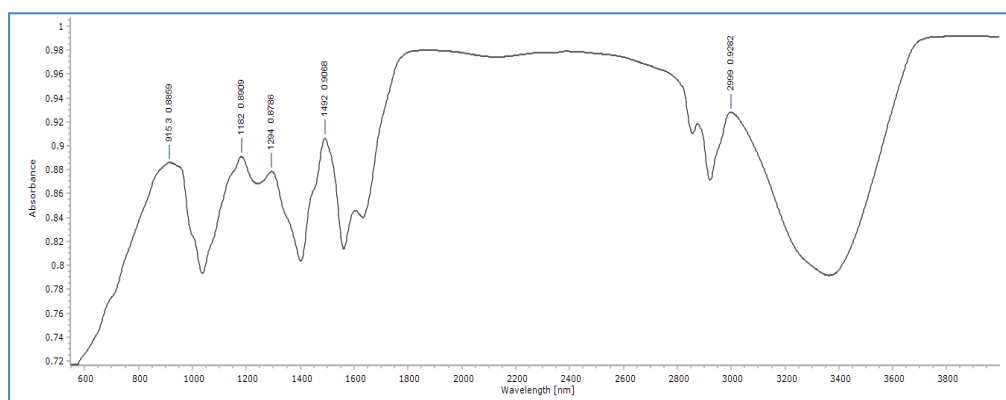


Fig. 7: FTIR spectral image of AuNPs.

stability, and band gap energy. A combined UV-Vis spectrum for mature leaves generated AuNPs at notable synthesised intervals is shown in Figure 3 for comparison with aqueous extract UV-Vis spectra. Figure 3 shows how the surface plasmon peak of the identical AuNPs changes over time. Because the green synthesis of gold nanoparticles may yield stable products, as evidenced by time dependent investigations, it is critical to get a mechanistic understanding of nanoparticle production. In the case of green synthesis, a reducing agent is employed (aqueous leaves extract). The precursor in this case is gold, which produces  $\text{Au}^+$  ion, which is reduced first to  $\text{Au}^{1+}$  (first reduction) and subsequently to  $\text{Au}^0$  (second reduction) by the citrate group. Essentially, the reduction is followed by the formation of clusters, which are subsequently transformed into bigger polycrystalline particles by aggregation.

According to one previous research, the growth process is explained in terms of the creation of 2.5 nm clusters, which are then organised into chains and networks of crystalline particles joined by 4 nm amorphous gold (Kamal, 2014; Unal *et al.*, 2020; Nutan *et al.*, 2020; Hussein *et al.*, 2020; Vijaykumar *et al.*, 2020). The chain's diameter grows until it collapses at 5-50 nm to form smaller spherical particles. Similarly, the growth of tiny particles continues in a cyclical way. SPR-dependent formation has also been demonstrated, including two to three stages such as nucleation, diffusion-controlled growth, and intraparticle ripening (Hu *et al.*, 2020; Muller *et al.*, 2021; Qiu *et al.*, 2021; Jakhu *et al.*, 2021; Jana *et al.*, 2021; Khatami *et al.*, 2021). The study with advanced instruments on particle growth suggests that the reaction temperature and the initial concentration of gold precursor are the factors

influencing the growth process, which significantly accelerate the particle formation process as the concentration of gold precursor increases. In the case of biogenesis (AuNP), the various phytochemicals contained in the *L. camara* aqueous extract, such as phenolic, alkaloid, flavonoid, enzymes, proteins, and so on, provide the first reduction step of  $Au^+$  to  $Au^0$ . They also serve as a stabilising agent for the newly created nanoparticles (Leen *et al.*, 2019).

Since no citrate is used for reduction, this is a green substance. Another type of growth process is chemical synthesis. By making the procedure inexpensive, it minimises the usage of harmful chemicals there. Polyphenols, organic acids, and proteins have well-established reducing capacities, thus the extract is predicted to have a synergistic impact with all of the phytochemicals. So far, no precise process for the production of plant extract-based nanoparticles has been discovered. However, the significance of

biosynthesis of nanoparticles using plant extract derives from the benefits of generating vast quantities without contamination and having a spherical form that is energetically favourable.

#### Antibacterial activity of AuNPs:

Table 2 shows that *Staphylococcus aureus* (25923) had a greater zone of inhibition (25 mm) in 40 mg/ml, while *Enterococcus faecalis* - 29212 had lower zone of inhibition (12 mm) in 40 mg/ml. ATCC culture shows activity in a dose-dependent manner whereas Table 3 showed *Staphylococcus aureus* alone had a greater zone inhibition (22mm) in 40 mg, while *Klebsiella pneumoniae* had lower zone of inhibition (13 mm) in 40 mg/ml when compared to other species of clinical strains of bacteria. Gold ions as well as AuNPs were known to have strong antibacterial activities. The antibacterial activity of different concentration of solutions containing AuNPs demonstrated that both Gram-positive and Gram-negative bacteria were inhibited. The inhibition rate at

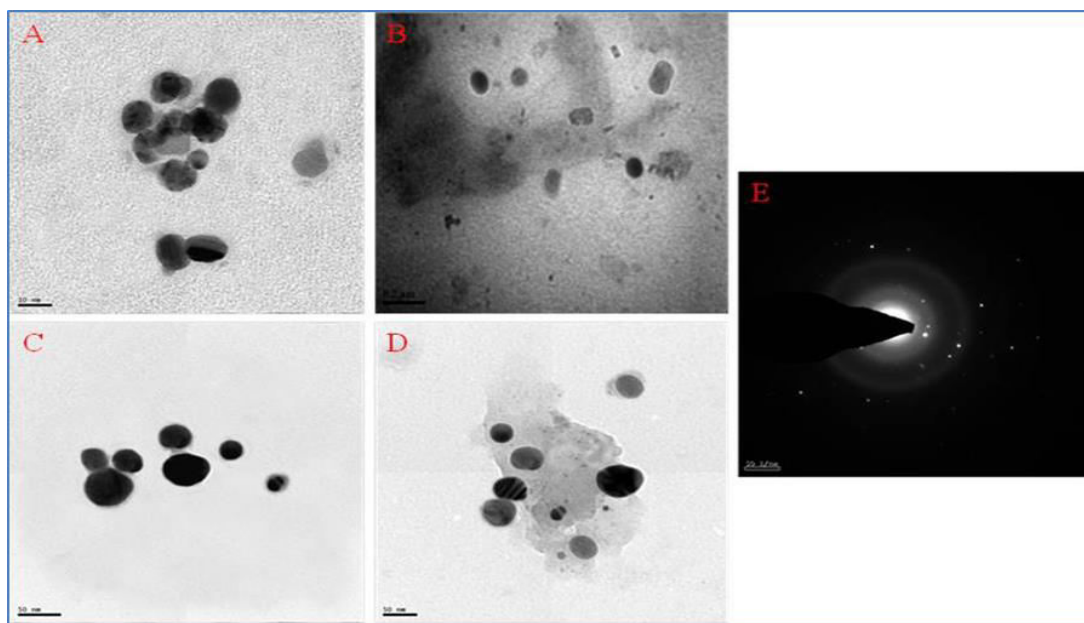


Fig. 8: HR-TEM image of *L. camara* AuNPs. (A): Ovoid shaped nanoparticles at 20 nm scale, (B): Bilayered ovoid shaped nanoparticles at 0.2 $\mu$ m scale, (C and D): slightly aggregated ovoid shape nanoparticles and (E): SADE image of polydispersive shaped nanoparticles.

Table 2: Antibacterial activity of ATCC culture against AuNPs

S. No.	Name of the organisms	Zone of Inhibition in mm				
		AK	10 mg	20 mg	30 mg	40 mg
1	<i>Escherichia coli</i> - 25922	14	11	16	17	18
2	<i>Klebsiella pneumoniae</i> - 13883	17	12	17	22	24
3	<i>Pseudomonas aeruginosa</i> -25873	28	12	15	17	20
4	<i>Enterobacter</i> - 200321	20	11	19	22	24
5	<i>Staphylococcus aureus</i> -25923	30	20	22	24	25
6	<i>Enterococcus faecalis</i> - 29212	12	10	12	14	15

Table 3: Antibacterial activity of clinical strains against AuNPs

S. No.	Name of the organisms	Zone of Inhibition in mm				
		AK	10 mg	20 mg	30 mg	40 mg
1	<i>Escherichia coli</i>	15	-	-	-	-
2	<i>Klebsiella pneumoniae</i>	19	09	11	12	13
3	<i>Pseudomonas aeruginosa</i>	18	-	-	-	-
4	<i>Enterobacter</i>	17	-	-	-	-
5	<i>Staphylococcus aureus</i>	19	15	17	19	22
6	<i>Enterococcus faecalis</i>	09	-	-	-	-

concentration of 40 mg/ml of AuNPs and the surface of bacteria cell membrane that disturbing the permeability, respiratory functions and finally leading to zone of inhibition were noticed in the present study.

AuNPs have many advantages such as simple and controlled synthesis, small size, and high biocompatibility and SPR, but whether they can be affected by *in vivo* or intracellular factors needs to be elucidated. Gold nanoclusters' small size and different shapes improve their antibacterial effects, but further research is necessary on the toxicity of organisms after a maximum concentration in our study. It is also indispensable to study the influence or changes in the use of AuNPs in a specific period in organisms or a defined physiological state in certain diseases. AuNPs are easily modified, and functionalized AuNPs have considerable antibacterial application potential. AuNPs can be modified to improve their antibacterial properties for clinical antibacterial

use. They can also be applied as drug carriers to improve drug efficacy, and their photothermal properties can be harnessed to kill bacteria. Combining AuNPs with different materials produces drugs with antibacterial properties. In summary, AuNPs' various characteristics can be used to functionalize them for specific requirements to provide new methods of solving different antibacterial challenges. These findings support the previous reports of antibacterial activity of AuNPs against *Bacillus cereus*, *Bacillus licheniformis* (Babu and Gunasekaran, 2009) and *Escherichia coli* (Kalimuthu *et al.*, 2008; Liu *et al.*, 2020)

## Conclusion

The green production of AuNPs utilising *L. camara* aqueous extract is presented in this study as an environmentally friendly technique. The extract indicates that the presence of various phytochemicals in the *L. camara* aqueous extract

contributes to the characteristics of both reducing and stabilising agents. The use of plant leaves maximises the use of undesired waste material while being economically friendly, efficient, and safe. There has been no prior research regarding the use of *L. camara* for the synthesis of AuNPs. The AuNPs that were created have the potential to be used in biomedical and other applications where nontoxicity is critical. AuNPs shows an active and stable antibacterial activity in both Gram-positive and Gram-negative bacteria, but it is more effective for Gram-positive organisms when compared with Gram-negative organisms. Because of its significant antibacterial efficacy, it may have potential applications in the field of biomedicine. Finally, it can be extendable to large scale production of Au NPs for commercial use.

## References

- Ajitha B, Reddy YAK and Reddy PS. (2015) Green synthesis and characterization of silver nanoparticles using *Lantana camara* leaf extract. *Materials Sci Engineer. C49*: 373-381.
- Aleixandre-Tudó JL., Bolaños-Pizarro M, Aleixandre JL and Aleixandre-Benavent R. (2020) Worldwide scientific research on nanotechnology: A bibliometric analysis of tendencies, funding, and challenges. *J Agricult Food Chem.* 68(34): 9158-9170.
- Anwar T, Qureshi H, Mahnashi MH, Kabir F, Parveen N, Ahmed D, Afzal U, Batool S, Muhammad A, Alyami SA and Alhaider HA. (2021) Bioherbicidal ability and weed management of allelopathic methyl esters from *Lantana camara*. *Saudi J Biol Sci.* 28(8): 4365-4374.
- Aritonang HF, Koleangan H and Wuntu AD. (2019) Synthesis of silver nanoparticles using aqueous extract of medicinal plants (*Impatiens balsamina* and *Lantana camara*) fresh leaves and analysis of antimicrobial activity. *Int J Microbiol.* 2019: 8642303.
- Babu G and Gunasekaran P. (2009) Production and structural characterization of crystalline silver nanoparticles from *Bacillus cereus* isolate. *Colloids Surf. B* 74:191-195.
- Bandi R, Dadigala R, Gangapuram BR and Guttena V. (2018) Green synthesis of highly fluorescent nitrogen-doped carbon dots from *Lantana camara* berries for effective detection of lead (II) and bioimaging. *J Photochem Photobiol B Biol.* 178: 330-338.
- Baruah S and Dutta J. (2009) Nanotechnology applications in pollution sensing and degradation in agriculture: a review. *Environ Chem Lett.* 7(3): 191-204.
- Bayda S, Adeel M, Tuccinardi T, Cordani M and Rizzolio F. (2020) The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine. *Molecules* 25(1): 112.
- Bhagwat SA, Breman E, Thekaekara T, Thornton TF and Willis KJ. (2012) A battle lost? Report on two centuries of invasion and management of *Lantana camara* L. in Australia, India and South Africa. *PLoS ONE* 7(3): e32407.
- Chowdhury R, Khan A and Rashid MH. (2020) Green synthesis of CuO nanoparticles using *Lantana camara* flower extract and their potential catalytic activity towards the aza-Michael reaction. *RSC Adv.* 10(24): 14374-14385.
- Currall SC, King EB, Lane N, Madera J and Turner S. (2006) What drives public acceptance of nanotechnology? *Nature Nanotech.* 1(3): 153-155.
- Delgado-Altamirano R, García-Aguilera ME, Delgado-Domínguez J, Becker I, de San Miguel ER, Rojas-Molina A and Esturau-Escofet N. (2021) 1H NMR profiling and chemometric analysis as an approach to predict the leishmanicidal activity of dichloromethane extracts from *Lantana camara* (L.). *J Pharmaceut Biomed Anal.* 199: 114060.
- Devi C and Khwairakpam M. (2020) Bioconversion of *Lantana camara* by vermicomposting with two different earthworm species in monoculture. *Bioresour Technol.* 296: 122308.
- DeRosa MC, Monreal C, Schnitzer M, Walsh R and Sultan Y. (2010) Nanotechnology in fertilizers. *Nature Nanotechnol.* 5(2): 91-91.
- El-Sayed A and Kamel M. (2020) Advanced applications of nanotechnology in veterinary medicine. *Environ Sci Poll Res.* 27(16): 19073-19086.
- Etuh MA, Ohemu LT and Pam DD. (2021) *Lantana camara* ethanolic leaves extracts exhibit anti-aging properties in *Drosophila melanogaster*: survival-rate and life span studies. *Toxicol Res.* 10(1): 79-83.
- Ghosh S, Das Sarma M, Patra A and Hazra B. (2010) Anti-inflammatory and anticancer compounds isolated from *Ventilago madraspatina* Gaertn., *Rubia cordifolia* Linn. and *Lantana camara* Linn. *J Pharm Pharmacol.* 62(9): 1158-1166.
- Gomez-Marquez J and Hamad-Schifferli K. (2021) Local development of nanotechnology-based diagnostics.

- Nature Nanotechnol. 16(5): 484-486.
- Gooden B, French K, Turner PJ and Downey PO. (2009) Impact threshold for an alien plant invader, *Lantana camara* L., on native plant communities. Biol Conserv. 142(11): 2631-2641.
- Gottardo S, Mech A, Drbohlavova J, Malyska A, Bøwadt S, Sintes JR and Rauscher H. (2021) Towards safe and sustainable innovation in nanotechnology: State-of-play for smart nanomaterials. NanoImpact 21: 100297.
- Guo Z and Cui Z. (2021) Fluorescent nanotechnology for in vivo imaging. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 13(5): e1705.
- Hu X, Xu X, Fu F, Yang B, Zhang J, Zhang Y, Touhid SSB, Liu L, Dong Y, Liu X and Yao J. (2020) Synthesis of bimetallic silver-gold nanoparticle composites using a cellulose dope: Tunable nanostructure and its biological activity. Carbohydrate Polymers 248: 116777.
- Hussein MA, El-Said WA, Abu-Zied BM and Choi JW. (2020) Nanosheet composed of gold nanoparticle/graphene/epoxy resin based on ultrasonic fabrication for flexible dopamine biosensor using surface-enhanced Raman spectroscopy. Nano Converg. 7(1): 1-12.
- Jakhu S, Sharma Y, Sharma K, Vaid K, Dhar H, Kumar V, Singh RP, Shekh A and Kumar G. (2021) Production and characterization of micro algal exopolysaccharide as a reducing and stabilizing agent for green synthesis of gold-nanoparticle: a case study with a *Chlorella* sp. from Himalayan high-altitude psychrophilic habitat. J Appl Phycol. 33: 3899-3914.
- Jana SK, Gucchait A, Paul S, Saha T, Acharya S, Hoque KM, Mishra AK, Chatterjee BK, Chatterjee T and Chakrabarti P. (2021) Virstatin-Conjugated Gold Nanoparticle with Enhanced Antimicrobial Activity against the *Vibrio cholerae* El Tor Biotype. ACS Appl Bio Mater. 4(4): 3089-3100.
- Joshi M, Bhattacharyya A and Ali SW. (2008) Characterization techniques for nanotechnology applications in textiles. Indian J Fibre Textile Res. 33: 304-317.
- Kalimuthu K, Babu RS, Venkataraman D, Bilal M and Gurunathan S (2008) Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloids Surf. B 65:150-153.
- Kamal A. (2014) Phytochemical screening of *Syzygium cumini* seeds. Indian J Plant Sci. 3(4): 1-4.
- Kato-Noguchi H and Kurniadie D. (2021) Allelopathy of *Lantana camara* as an invasive plant. Plants 10(5): 1028.
- Khatami M, Mosazade F, Raeisi M, Ghasemi M, Fazli Z, Arefkia K, Varma RS, Borhani F and Khatami S. (2021) Simplification of gold nanoparticle synthesis with low cytotoxicity using a greener approach: opening up new possibilities. RSC Adv. 11(6): 3288-3294.
- Kumar B, Kumari S, Cumbal L and Debut A. (2015) *Lantana camara* berry for the synthesis of silver nanoparticles. Asian Pacific J Trop Biomed. 5(3): 192-195.
- Kumar S, Sandhir R and Ojha S. (2014) Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. BMC Res Notes 7(1): 1-9.
- Lateef A, Gueguim-Kana EB, Dasgupta N and Ranjan S. (2021). Microbial nanobiotechnology: Principles and applications. Springer.
- Leen Othaman, Ahmad S and Roula MAM (2019) Antimicrobial activity of polyphenols and alkaloids in the middle eastern plants. Front Microbiol. 10: 991.
- Liu Q, Chen, L, Laserna AKC, He Y, Feng X and Yang H. (2020) Synergistic action of electrolyzed water and mild heat for enhanced microbial inactivation of *Escherichia coli* O157:H7 revealed by metabolomics analysis. Food Control. 110: 107026.
- Liu S, Ali S, Yang R, Tao J and Ren B. (2019) A newly discovered Cd-hyperaccumulator *Lantana camara* L. J Hazard Mater. 371: 233-242.
- Macedo DCS, Almeida FJE, Wanderley MSO, Ferraz MS, Santos NPS, López AMQ, Santos-Magalheas NS and Lira-Nogueira MCB. (2021) Usnic acid: from an ancient lichen derivative to promising biological and nanotechnology applications. Phytochem Rev. 20: 609-630.
- Mitter N and Hussey K. (2019) Moving policy and regulation forward for nanotechnology applications in agriculture. Nature Nanotechnol. 14(6): 508-510.
- Müller AK, Xu ZK and Greiner A. (2021) Preparation and performance assessment of low-pressure affinity membranes based on functionalized, electrospun polyacrylates for gold nanoparticle filtration. ACS Appl Mater Interfaces 13(13): 15659-15667.
- Naz R and Bano A. (2013) Phytochemical screening, antioxidants and antimicrobial potential of *Lantana camara* in different solvents. Asian Pac J Trop Dis. 3(6): 480-486.
- Nutan B, Chandel AKS, Biswas A, Kumar A, Yadav A, Maiti P and Jewrajka SK. (2020) Gold nanoparticle

- promoted formation and biological properties of injectable hydrogels. *Biomacromolec.* 21(9): 3782-3794.
- Ogendo JO, Belmain SR, Deng AL and Walker DJ. (2003) Comparison of toxic and repellent effects of *Lantana camara* L. with *Tephrosia vogelii* Hook and a synthetic pesticide against *Sitophilus zeamais* Motschulsky (Coleoptera:Curculionidae) in stored maize grain. *Int J Trop Isect Sci.* 23(2): 127-135.
- Park J, Jeong BG, Kim SI, Lee D, Kim J, Shin C, Lee CB, Otsuka T, Kyoung J, Kim S, Yang KY, Park YY, Lee J, Hwang I, Jang J, Song SH, Brongersma ML, Ha K, Hwang SW, Choo H and Choi BL. (2021) All-solid-state spatial light modulator with independent phase and amplitude control for three-dimensional LiDAR applications. *Nature Nanotechnol.* 16(1): 69-76.
- Pereira JM, Barreto RW, Ellison CA and Maffia, LA. (2003) *Corynespora cassiicola* f. sp. *lantanae*: a potential biocontrol agent from Brazil for *Lantana camara*. *Biol Cont.* 26(1): 21-31.
- Qamar F, Begum S, Raza SM, Wahab A and Siddiqui BS. (2005) Nematicidal natural products from the aerial parts of *Lantana camara* Linn. *Nat Product Res.* 19(6): 609-613.
- Qiu R, Xiong W, Hua W, He Y, Sun X, Xing M and Wang L. (2021) A biosynthesized gold nanoparticle from *Staphylococcus aureus* - as a functional factor in muscle tissue engineering. *Appl Mater Today* 22: 100905.
- Rakonjac J, Bennett NJ, Spagnuolo J, Gagic D and Russel M. (2011) Filamentous bacteriophage: biology, phage display and nanotechnology applications. *Curr Issues Molec Biol.* 13(2): 51-76.
- Rana KL, Kour D, Yadav N and Yadav AN. (2020) Endophytic microbes in nanotechnology: current development, and potential biotechnology applications. In: *Microbial Endophytes*, Woodhead Publishing, pp. 231-262.
- Salamanca-Buentello F and Daar AS. (2021) Nanotechnology, equity and global health. *Nature Nanotechnol.* 16(4): 358-361.
- Sathish R, Vyawahare B and Natarajan K. (2011) Antiulcerogenic activity of *Lantana camara* leaves on gastric and duodenal ulcers in experimental rats. *J Ethnopharmacol.* 134(1): 195-197.
- Scheunert G, Heinonen O, Hardeman R, Lapicki A, Gubbins M and Bowman RM. (2016) A review of high magnetic moment thin films for microscale and nanotechnology applications. *Appl Phys Rev.* 3(1): 011301.
- Sonibare OO and Effiong I. (2008) Antibacterial activity and cytotoxicity of essential oil of *Lantana camara* L. leaves from Nigeria. *African J Biotechnol.* 7(15): 2618-2620.
- Svendsen C, Walker LA, Matzke M, Lahive E, Harrison S, Crossley A, Park B, Lofts S, Lynch I, Vázquez-Campos S, Kaegi R, Gogos A, Asbach C, Cornelis G, von der Kammer F, van den Brink NW, Mays C and Spurgeon DJ. (2020) Key principles and operational practices for improved nanotechnology environmental exposure assessment. *Nature Nanotechnol.* 15(9): 731-742.
- Swamy MK, Sinniah UR and Akhtar M. (2015) In vitro pharmacological activities and GC-MS analysis of different solvent extracts of *Lantana camara* leaves collected from tropical region of Malaysia. *Evidence-Based Complem Altern Med.* 2015: 506413.
- Tadesse E, Engidawork E, Nedi T and Mengistu G. (2017) Evaluation of the anti-diarrheal activity of the aqueous stem extract of *Lantana camara* Linn (Verbenaceae) in mice. *BMC Complem Altern Med.* 17(1): 1-8.
- Unal IS, Demirbas A, Onal I, Ildiz N and Ocsoy I. (2020) One step preparation of stable gold nanoparticle using red cabbage extracts under UV light and its catalytic activity. *J Photochem Photobiol B Biol.* 204: 111800.
- Verma RK and Verma SK. (2006) Phytochemical and termiticidal study of *Lantana camara* var. *aculeata* leaves. *Fitoterapia* 77(6): 466-468.
- Vijayakumar S, Vinayagam R, Anand MAV, Venkatachalam K, Saravanakumar K, Wang MH, Sangeetha CC, Gothandam KM and David E. (2020) Green synthesis of gold nanoparticle using *Ecliptaalba* and its antidiabetic activities through regulation of Bcl-2 expression in pancreatic cell line. *J Drug Delivery Sci Technol.* 58: 101786.
- Vijilvani C, Bindhu MR, Frincy FC, AlSalhi MS, Sabitha S, Saravanakumar K and Atif M. (2020) Antimicrobial and catalytic activities of biosynthesized gold, silver and palladium nanoparticles from *Solanum nigrum* leaves. *J Photochem Photobiol B Biol.* 202: 111-713.
- Wiesner MR and Bottero JY. (2017) *Environmental nanotechnology: Applications and impacts of nanomaterials.* McGraw-Hill Companies. <https://www.accessengineeringlibrary.com/content/book/9780071477505>.