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Original Article

Synthesis of Plant Mediated gold Nanoparticles using *Azima Tetracantha* Lam. Leaves extract and Evaluation of their Antimicrobial Activities

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ABSTRACT

Aim: The demand for nanoparticles is increasing day by day due to their wide range of applications in various areas including pharmaceutical industry. Nanoparticles are formally synthesized by chemical methods in which the toxic and flammable chemicals are used. **Methods:** This article reports about an effective, rapid and eco-friendly technique for the fabrication of gold nanoparticles from gold chloride solution using *Azima tetracantha* Lam. leaves extract. The effects of the leaves extract of *Azima tetracantha*, the concentration of Gold chloride solution, the time of the reaction and the effect of tem¬perature on the rate of the reaction were investigated. The synthesized gold nanoparticles (AuNPs) were characterized by using various techniques such as Dynamic Light Spectroscopy (DLS), Scanning Electron Microscopy (SEM), UV-Vis spectra gave surface plasmon resonance (SPR) at 540 nm, Fourier Transform Infrared spectroscopy (FTIR) and X-ray diffraction (XRD). This revealed the reduction of gold ions (Au^{+}) to gold metal (Au^{0}) which indicated the formation of gold nanoparticles (AuNPs). **Results:** The antimicrobial action of biosynthesized AuNPs indicated effective activity against bacterial pathogens *Aeromonas liquefaciens*, *Enterococcus fecalis*, *Micrococcus luteus*, *Salmonella typhimurium* and fungal pathogens *Candida albicans*, *Cryptococcus sp*, *Microsporum canis*, *Trichophyton rubrum*. **Conclusion:** This revealed that gold nanoparticles could provide a safer alternative to conventional antimicrobial agents.

Key words: Gold Nanoparticles, *Azima tetracantha* Leaves Extract, Biosynthesis, Characterization, Antimicrobial Activity.

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INTRODUCTION

Indian flora is the chief and cheap source of medicinal plants and plant products. For many decades, these medicinal plants have been widely utilized in Ayurveda. Nowadays, many such plants are gaining importance due to their unique bioactive constituents and their versatile applicability in various fields of research and development.

The recent developments in nanotechnology have influenced all types of life. With the advancement of technologies a completely unique approach emulated for analysis and development within plant biology field, medicine and nanotechnology, such as development in using plants or their elements for the inexperienced synthesis of nanoparticles. Nanotechnology could be a staggeringly developing powerful technology that holds an enormous promise for the development of many novel products with its potential medical applications on early illness detection, treatment, and hindrance. New technologies often create new challenges to science in addition to their benefits, raise concerns about health and various environmental problems. Recent nanotechnology holds a promise and a broad aspect towards wide applications of nanoparticles in a multiple way of emerging fields of science and technology.

Over last decades, nanotechnology has established as the great innovation of science and technology. A number of methods are available for the fabrication of nanoparticles mainly chemical synthesis like reduction in solutions, physical synthesis like thermal decomposition, radiation assisted process and recently through green chemistry route or biological synthesis using plants, bacteria and fungi. Most of the chemical and physical methods of synthesis of nanoparticles can control the size and shape of nanoparticles. Therefore, a biological process with the ability to perform the same has been an exciting prospect.

Various microorganisms are exploited for the biosynthesis of nanoparticles but recently a new trend has come to force i.e., the use of plants and plant products for the synthesis of nanoparticles because of its spontaneous, cost effective, environmental-friendly protocol, suitable for large scale production.[1] The main mechanism considered for the synthesis of nanoparticles mediated by the plants is due to the presence of phytochemicals. The major phytochemicals responsible for the spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides. Many research works are available on the biosynthesis of nanoparticles using plants and plant leaves extract, such as *Ficus benghalensis*,[2] *Rosa rugosa*,[3] *Stevia rebaudiana*,[4] *Chenopodium album*,[5] *Nicotiana tobaccum*,[6] *Trianthema decandra*,[7] *Polyalthia longifolia*,[8] *Cycas species*,[9] *Pinus desiflora*, *Diopyros kaki*, *Ginko biloba*, *Magnolia kobus*, and *Pllatanus orientalis*,[10] *Catharanthus roseus*,[11] *Pungamia pinnata*, *Hemidesmus indicus*, *Syzygium cumini*, *Allium cepa*, and *Pandaanus odorifer*,[12] *Sesuvium portulacastrum* L,[13] *Acalypha indica*,[14] *Parthenium hysterophorous*,[15] *Capsicum annuum*,[16] *Piper longum*,[17] *Arbutus unedo*,[18] *Ocimum santum*,[19] and *mulberry species*.[20] On the basis of available scientific literature, this methodology was designed with a very simple, quick, efficient and environmental friendly method for gold nanoparticles synthesis at appropriate conditions using *Azima tetracantha* leaves extract.

MATERIALS AND METHODS

Preparation of *Azima tetracantha* Leaves Extract

Fresh leaves of *Azima tetracantha,* Figure 1 were collected from Keelapaluvur village (Latitude–11^{0}02'27.56" N; Longitude–79^{0}04'08.08" E), Ariyalur district of Tamil Nadu in India. No specific permissions were required for collecting these leaves as these plants grow commonly in our village as well as this field studies did not involve endangered or protected species. Fresh *A. tetracantha* leaves were individually collected, thoroughly washed with running tap water and then with double distilled water, up to 5 days shade-dried and by using mechanical grinder a fine powder was prepared. 20 g of the fine leaf powder was mixed with 200 ml of pre-sterilized Milli Q water. The mixture was boile dat 60°C for 10 min to allow the conversion by boiling water bath. What man no.1 filter paper were used to filter the extract and stored in a refrigerator at 4°C for further studies.

Gold nanoparticles synthesis[21]

Gold chloride was prepared at the 10^{-3} M concentration with pre-sterilized Milli Q water. 10 ml of the leaf extract was mixed with 90 ml of 10^{-3} M gold chloride for the synthesis of gold nanoparticles. Gold chloride was taken in similar quantities without adding leaf extracts to main respective controls. The containers were covered tightly with aluminium foil to prevent photo reduction of gold ions, and thereby incubated under dark condition at room temperature and observations were recorded. The colour of the solution mixture of gold chloride and *Azima tetracantha* leaves extract changed from pale yellow colour to dark pink colour at 60°C and 10 min of reaction time. This indicated the reduction of gold metallic (Au^+) ions to gold (Au^0) nanoparticles.

Characterization of gold Nanoparticles

UV-Vis Spectroscopy[22]

The optical property of biosynthesized gold nanoparticles samples were measured at room temperature by UV-Vis spectrophotometer (Perkin-Elmer) operated at 1 nm between 200 and 800 nm range of resolution.

Fourier Transform Infrared Spectroscopy (FTIR)

The characterization of functional groups on the surface of AuNPs by leaf extracts were investigated by FTIR analysis (Shimadzu) and the spectra were scanned in the range of 4000–400 cm^{-1} ranges at a resolution of 4 cm^{-1}. The samples were prepared by dispersing the Au NPs uniformly in a matrix of dry KBr, compressed to form transparent disc. KBr was used as a standard to analyse the samples.

Scanning Electron Microscopy (SEM)

SEM analysis of gold nanoparticles was done using Jeol JSM-5800 SEM machine. a very small amount of the sample was droppe on a carbon coated copper grid and thin films of the gold nanoparticles were prepared. The thin films were allowed to dry the sample was analysed.

X-ray diffraction measurements (XRD)

XRD measurements of the reduced Au NPs were recorded on X-ray diffractometer (x'pert pananalytical) instrument operating at a current of 30 mA with Cu K (α) radiation voltage of 40 kV to determine the crystalline phase and material identification. The samples were taken in lids and put under instrument for analysis. All the data were collected in the angular range $30 \leq 2\theta \leq 500$, under the same experimental conditions,

Antimicrobial Studies

Antimicrobial activity of the biosynthesized gold nanoparticles against human bacterial pathogens *Aeromonas liquefaciens* (B1), *Enterococcus fecalis* (B2), *Micrococcus luteus* (B3), *Salmonella typhimurium* (B4) and fungal pathogens *Candida albicans* (F1), *Cryptococcus sp* (F2), *Microsporum canis* (F3), *Trichophyton rubrum* (F4) were determined by using agar-well diffusion assay.[23] Aqueous dispersions of gold nanoparticles of two different concentrations[12,22] 15 µL and 30 µL were made. Methicillin at a concentration of 10 mcg/disc and Itraconazole at a concentration of 10 mcg/disc were taken as positive controls for bacteria and fungi respectively.

RESULTS AND DISCUSSION

UV-Vis Spectral Study

Biosynthesized leaves extract mediated AuNPs particles were confirmed using UV–Vis spectrophotometer by analysing the excitation due to the applied electromagnetic field of surface plasmon resonance (SPR) at 540 nm and the peak was observed between 535-550 nm. Figure 2 shows the UV absorption peaks of *A. tetracantha*. It clearly indicated the formation of AuNPs of the leaves extract of the plant. The change in colour is due to the excitation of surface plasmon vibration, which is indicated by the reduction of Au^+ ions to Au^0 ions at different time intervals. During each interval of time, the peak became clear, unique and rising. This unique peak clearly indicates the increase in synthesis of nanoparticles as the time increases. Similarly, the colour also became intensified as the time increases. Similar results were observed by some other researchers.[24,25] Jayaseelan *et al*, 2013[26] has reported that aqueous extract of *Abelmoschus esculentus seeds* showed the SPR at 536 nm.

FTIR spectrum of *Azima tetracantha* Leaves Extract mediated gold nanoparticles

To determine the functional groups of *Azima tetracantha* leaves extract, a FT-IR analysis was done and the results were shown in Figure 3. The *Azima tetracantha* leaves extract exhibited a number of absorption peaks, reflecting its complexity in nature. FT-IR analysis revealed the strong bands at 3306, 2124, and 1637 cm^{-1}. The band at 3306 cm^{-1} corresponds to NH-amine amino groups, 2124 alkane C-H stretching-lipids, 1637 cm^{-1} corresponds to amide amino groups. FT-IR analysis of the *Azima tetracantha* leaves extract indicated that the carboxyl groups (-C=O), hydroxyl groups (-OH) and amine (N-H) groups of *Azima tetracantha* leaves extract are mainly involved in reduction of Au^+ to Au^0 nanoparticles. Same results were reported by other researchers and reported that wave numbers signal stretching and vibrational bending of the peaks may be derived from compounds such as flavonoids, terpenoids, alkaloids and soluble proteins present in plants extracts and these may be responsible for the stabilization of gold nanoparticles.[27]

Scanning Electron Microscopy (SEM)

SEM analysis of the products was recorded and the synthesised gold nanoparticles are found to be spherical in structure of about 80 nm in diameter (Figure 4). The SEM image showed the formation of gold nanoparticles using *A. tetracantha* leaves extract and confirmed the development of gold nanostructures. The same results were observed by Sobczak-Kupiec *et al.*[28]

X-ray diffraction measurements (XRD)

Gold nanoparticles were synthesized using leaves of *A. tetracantha* and the synthesis was confirmed by observable colour change in the mixture and also confirmed by UV-VIS spectrum. Subsequently, XRD analysis

Table 1: Antimicrobial screening of AuNPs derived by *Azima tetracantha* leaves

Test Microorganisms	Zone of inhibition (mm) Sample (15 & 30) µL / disc				Diseases	Route of Transmission
	Bacteria	15 µL	30 µL	PC	Remarks	
Aeromonas liquefaciens B1	16	18	14	> PC	Wound Infections /Gastroenteritis	Water/Food
Enterococcus fecalis B2	18	19	8	> PC	Endocarditis /Bladder, Prostate, and Epididymal Infections/Nervous system Infections	Water/Food
Micrococcus luteus B3	19	20	38	< PC	Skin and Pulmonary infections/ Septic shock/ Pneumonia endocarditis	Soil/Dust/Water/Airways/ Food
Salmonella typhimurium B4	16	17	0	> PC	Typhoid	Water/Food
	Fungi					
Candida albicans F1	12	14	10	> PC	Skin (Integument) Infections /Gastrointestinal tract Infection	Airways/Wound/Soil/Water
Cryptococcus sp. F2	10	11	9	> PC	Cryptococcal disease/Bronchiectasis/ Endophthalmitis.	Airways/Wound/Soil/Water
Microsporum canis F3	12	16	9	> PC	Tinea capitis/Ringworm	Airways/Wound/Soil/Water
Trichophyton rubrum F4	11	14	7	> PC	Tinea corporis/Tinea cruris/Tinea pedis/ Onychomycosis	Airways/Wound/Soil/Water

PC -Positive Control; (Bacteria–Methicillin (10 mcg/disc); Fungi–Itraconazole (10 mcg/disc); > PC–greater than positive control; < PC–less than positive control.

Figure 1: A Twig of *Azima tetracantha* Lam

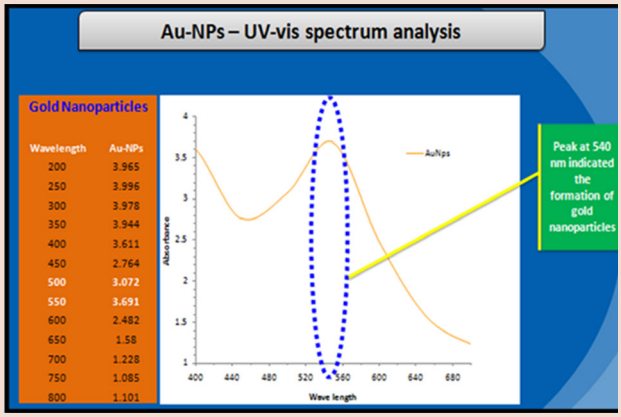

Figure 2: Au-NPs- UV- Vis Spectrum Analysis.

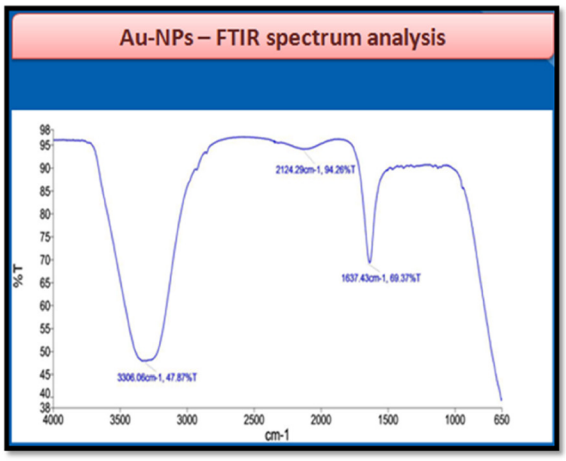

Figure 3: Au-NPs- FTIR Spectrum Analysis.

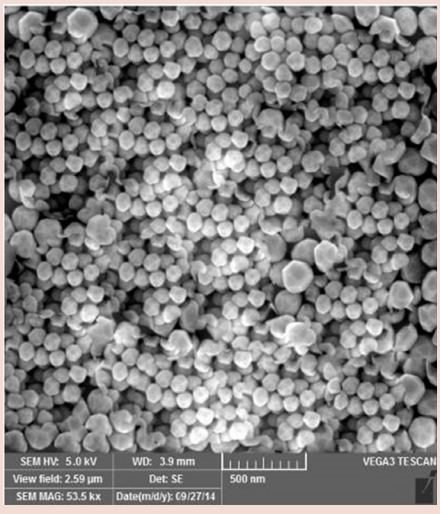

Figure 4: SEM Image of AT Leaves Mediated Au-NPs.

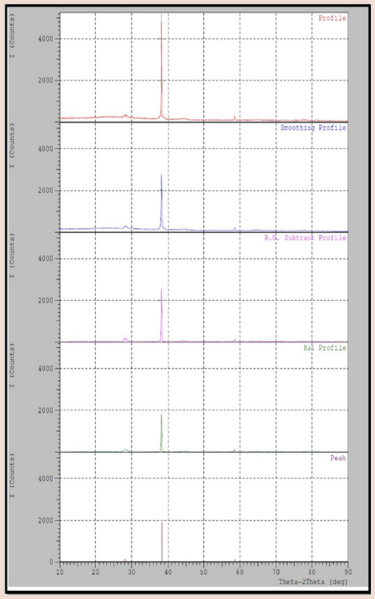

Figure 5: X-ray diffraction measurements.

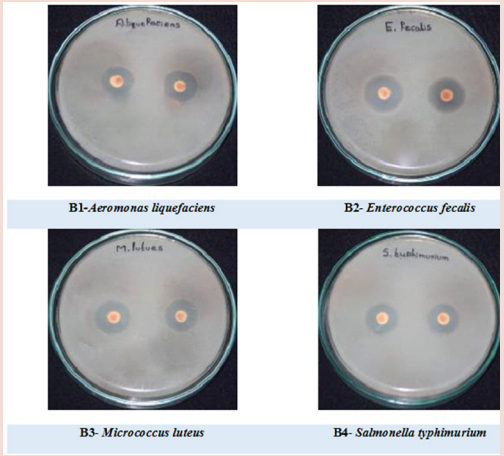

Figure 6: Antibacterial Studies.

was used to analyse the phase distribution, crystallinity and purity of the synthesised nanoparticles. X-ray diffraction (XRD) pattern of synthesized particles were analysed and peak profile of relevant particles were found.

In Figure 5, a peak was observed at range of 2θ values of 38 which corresponded to the Bragg's reflections such as 111, 200, 220 and 311. Similar peaks and lattice planes were observed by other researchers also.[24,25]

Antimicrobial Studies

The antimicrobial activity of the antibiotics (Positive control-Methicilin and Itraconazole) and *Azima tetracantha* leaves extract mediated gold nanoparticles were challenged against various NCIM and MTCC microbes using agar well diffusion assay. The test concentrations (15 and 30 µl/disc) produced zone of lysis on MHA and PDA plates for bacteria and fungi respectively. *Azima tetracantha* leaves extract mediated gold nanoparticles were most effective against *Salmonella typhimurium* NCIM 2501 (B4) while minimal effect was observed from *Micrococcus luteus* NCIM 2871 (B3) in the bacterial division. But in fungi, which

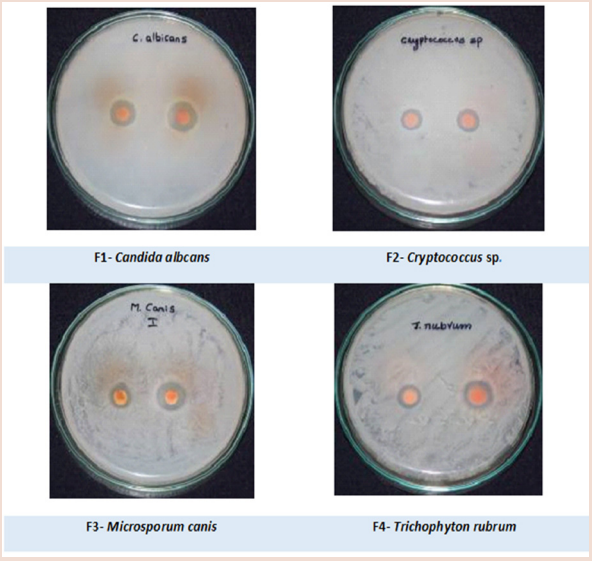

Figure 7: Antifungal Studies.

was effective against *Trichophyton rubrum* MTCC 3272 (F4) produced a minimal smaller effect in *Cryptococcus* sp. MTCC 7076 (F2) (Figure 6 and Figure 7). The higher (30 µL/disc) concentration showed larger zone effect than the lower (15 µL/disc) concentration against certain microorganisms. All the microbial strains exhibited higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control except B3 (Table 1). Grace and Pandian[29] also reported a great bactericidal effect of gold nanoparticles and the well-developed chemical stability, appropriate smaller size make gold nanoparticles and surface chemistry easier to interact with the microorganisms.

CONCLUSION

This study advanced a fast, effective, convenient biological method and environmental-friendly for the fabrication of stabilized gold nanoparticles of average diameter of 80 nm using the leaves extract of *Azima tetracantha*. The formation of gold nanoparticles was confirmed and characterized by UV-vis, FT-IR, XRD and SEM analytical methods. The antimicrobial activity of biologically synthesized leaves mediated gold nanoparticles was evaluated against human bacterial pathogens *Aeromonas liquefaciens*, *Enterococcus fecalis*, *Micrococcus luteus*, *Salmonella typhimurium* and fungal pathogens *Candida albicans*, *Cryptococcus sp*, *Microsporum canis*, *Trichophyton rubrum* showing effective antimicrobial activity.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: AH, NB, MI Performed the experiments: AH Analysed the data: AH, NB, MI, and IQ Contributed reagents/materials/analysis tools: AH, PK, IQ Contributed to the writing of the manuscript: AH, SM, IQ.

CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATION USED

AT: *Azima tetracantha*; **AuNPs:** Gold Nanoparticles; **UV-Vis:** Ultraviolet-Visible Spectroscopy; **SEM:** Scanning Electron Microscopy; **XRD:** X-Ray Diffraction; **DLS:** Dynamic Light Spectroscopy; **FTIR:** Fourier Transform Infrared Spectroscopy; **SPR:** Surface Plasmon Resonance; **KBr:** Potassium Bromide; **NCIM:** National Collection of Industrial Microorganisms, **MTCC:** Microbial Type Culture Collection; **MHA:** Muller Hinton Agar; **PDA:** Potato Dextrose Agar; **PC:** Positive Control.

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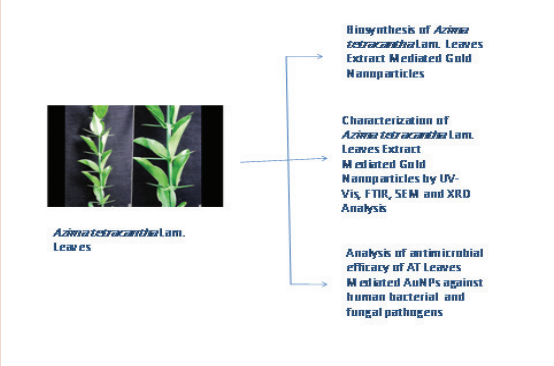
PICTORIAL ABSTRACT



SUMMARY

- A fast, effective, environmental-friendly and convenient biological method for the fabrication of stabilized gold nanoparticles was developed using the leaves extract of *Azima tetracantha*.
- The formation of gold nanoparticles was confirmed and characterized by UV–vis, FT-IR, XRD and SEM analytical methods.
- The antimicrobial activity of biologically synthesized leaves mediated gold nanoparticles was evaluated against human bacterial pathogens *Aeromonas liquefaciens*, *Enterococcus fecalis*, *Micrococcus luteus*, *Salmonella typhimurium* and fungal pathogens *Candida albicans*, *Cryptococcus sp*, *Microsporum canis*, *Trichophyton rubrum*.
- *Azima tetracantha* leaves extract mediated gold nanoparticles were most effective against *Salmonella typhimurium* while minimal effect was observed from *Micrococcus luteus*.
- In fungi, it was effective against *Trichophyton rubrum* but produced a minimal effect in *Cryptococcus* sp.

ABOUT AUTHORS

Abirami: Is a doctoral student in the P.G. and Research Department of Biotechnology, Jamal Mohamed College, Tiruchirappalli, Tamil Nadu, India. Her doctoral research focused on the nanoparticles synthesis and evaluation of anticancer and antimicrobial efficacy of the leaves extracts of *Azima tetracantha* Lam. both *in vitro* and *in vivo*.

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