ISSN: 2349 - 4891

International
Journal of Recent Research and Applied Studies
(Multidisciplinary Open Access Refereed e-Journal)

Plectranthus amboinicus derived nanoparticles for Antimicrobial activity: A case study of gold nanoparticles synthesis and characterizations

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Received 19th March 2020, Accepted 1st May 2020

Abstract

In this present study, biofabrication and characterization of gold nanoparticles (AuNPs), and their application on human pathogenic bacteria and fungus were investigated. The AuNPs was prepared by the method of green synthesis using the extract of *P. amboinicus*. The formation and characterization of AuNPs were confirmed by UV-Vis spectroscopy, Fourier Infrared Spectroscopy (FTIR) energy-dispersive spectroscopy (EDX), X-ray diffraction (XRD) and Scanning electron microscope (SEM). The AuNPs were challenged against certain pathogenic bacterial and fungal strains. In antimicrobial activity, the AuNPs was most effective against *Salmonella typhimurium* while smaller effect was noticed from *Micrococcus luteus*.

Keywords: Biofabrication, Gold nanoparticles, *P. amboinicus*, Antimicrobial Activity.

Introduction

The field of nanotechnology is one of the most active research areas in modern materials science. Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology (Hussain Beevi et al., 2012). There have been impressive developments in the field of nanotechnology in the recent past years, with numerous methodologies developed to synthesize nanoparticles of particular shape and size depending on specific requirements. New applications of nanoparticles and nano materials are increasing rapidly (Vignesh et al., 2014). Recently, metal nanoparticles have gained a lot of attention due to their unique chemical, optical, magnetic, mechanical and electric magnetic properties. Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100 nm) materials for the development of science (Huang, 2004).

Nanotechnology is also being utilized in medicine for diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders (Anitha et al., 2011). Nanoparticles rapidly interact with the proteins present in various biological systems. In this paper we report, for the first time to the best of our knowledge, biosynthesis of the Au nanoparticles using *P. amboinicus* leaf extract as a reducing agent and demonstrate that this method yields faster and stable gold nanoparticles compared to other methods. Our studies reveal that the gold nanoparticles demonstrate antimicrobial activities were carried out against microbial strains by using disc diffusion method (Ahmed John and Koperuncholan, 2012a).

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Materials and methods

Study area and sampling

The plant materials were collected from Perambalur District of Tamil Nadu in India during the period of January to February 2019. The shade dried *P. amboinicus* fine powder was sterilized at 121°C for 15 min and weighed. Sterilized fine powder, 20 g each was taken each plant, mixed with 200 ml of Milli Q water and kept in boiling water bath at 60°C for 10 min. The extracts were filtered with Whatman 1 filter paper and the filtered extracts were stored in a refrigerator at 4°C and it's used as test samples.

Biofabrication of nanoparticles

Biofabrication of gold nanoparticles, gold chloride prepared at the concentration of 10^{-3} M with pre-sterilized Milli Q water. A quantity of 10 ml plant extract was mixed with 90 ml of 10^{-3} M gold chloride for the synthesis of gold nanoparticles. Gold chloride has taken in similar quantities without adding plant extracts to main respective controls. The saline bottles were tightly covered with aluminium foil in order to avoid photo reduction of gold ions, incubated at room temperature under dark condition and observations were recorded.

Characterization of nanoparticles

UV-VIS spectroscopy

The Au nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer, Lambda-19 to know the kinetic behaviour of Au nanoparticles. The scanning range of the samples was 200-800 nm at a scan speed of 480 mm/min. Baseline correction of the spectrophotometer was carried out by using a blank reference.

Fourier transform-infra red (FT-IR) spectroscopy

The analysis of bio-reducing agent present in each of the extracts was measured by FT-IR. After the reaction, a small aliquot of the concentrated reaction mixture was measured in the transmittance mode at 400 to 4000 cm^{-1}. The spectra of the extracts taken after the biosynthesis of nanoparticles were analyzed.

Scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS)

In this research work, Joel JSM-6480 LV SEM machine was used to characterize the mean particle size and morphology of nanoparticles. Compositional analysis on the sample was carried out by the energy dispersive X-ray spectroscopy (EDS) attached with the SEM. The EDS analysis of Ag sample was done by the SEM (JEOLJSM 5800) machine. The EDS normally reveals the presence of phases.

X-ray diffraction method

The phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique (Philips PAN analytical, The Netherlands) using Cu radiation. The generator voltage and current was set at 40 KV and 30 mA respectively. The Au sample was scanned in the range 10.0000 - 90.0000^{o} in continuous scan mode. The scan rate was 0.60/sec.

Testing of antimicrobial activity

The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus* sp. MTCC 7076 (F2), *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains were tested for antimicrobial sensitivity using the plate diffusion method. (Koperuncholan and Ahmed John, 2011a; Pandiyarajan et al., 2013; Lakshmi praba et al., 2013). The antibacterial and antifungal activities of test samples were analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively (Vignesh et al., 2012a; Vignesh et al., 2013). A sterile cotton swab was used to inoculate the bacterial suspension on surface of agar plate. The two different concentrations (50 and 100µl) of gold nano samples were poured into well (1 cm in diameter and 4 mm in depth) of the agar plates, separately (Ahmed John and Koperuncholan**,** 2012b). The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungi). After incubation, the zone of inhibition was measured with ruler/ antibiotic zone scale-C (Vignesh et al., 2012b). The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control. All the media, standard discs and sterile disc were purchased from Hi-Media (Mumbai, India).

Result and discussion

Chloroauric acid was selected for the study because of their high antibacterial property and commercially more viable. Leaves of *P. amboinicus* (medicinal plants) leaves was selected for the study of biosynthesis of gold nanoparticles.

Biosynthesis of gold nanoparticles (AuNPs)

When the *P. amboinicus* leaves fine powder aqueous extract was mixed at 0.1% concentration of chloroauric acid ($HAuCl_4$) aqueous solutions the solutions changed their color from pale brown to pink for gold nanoparticles (Figure 1 & 2). The change in color is due to the excitation of surface plasmon vibration, which is indicated by the formation of gold nanoparticles at different time intervals. Spectroscopic data were analyzed to characterize gold nanoparticles.

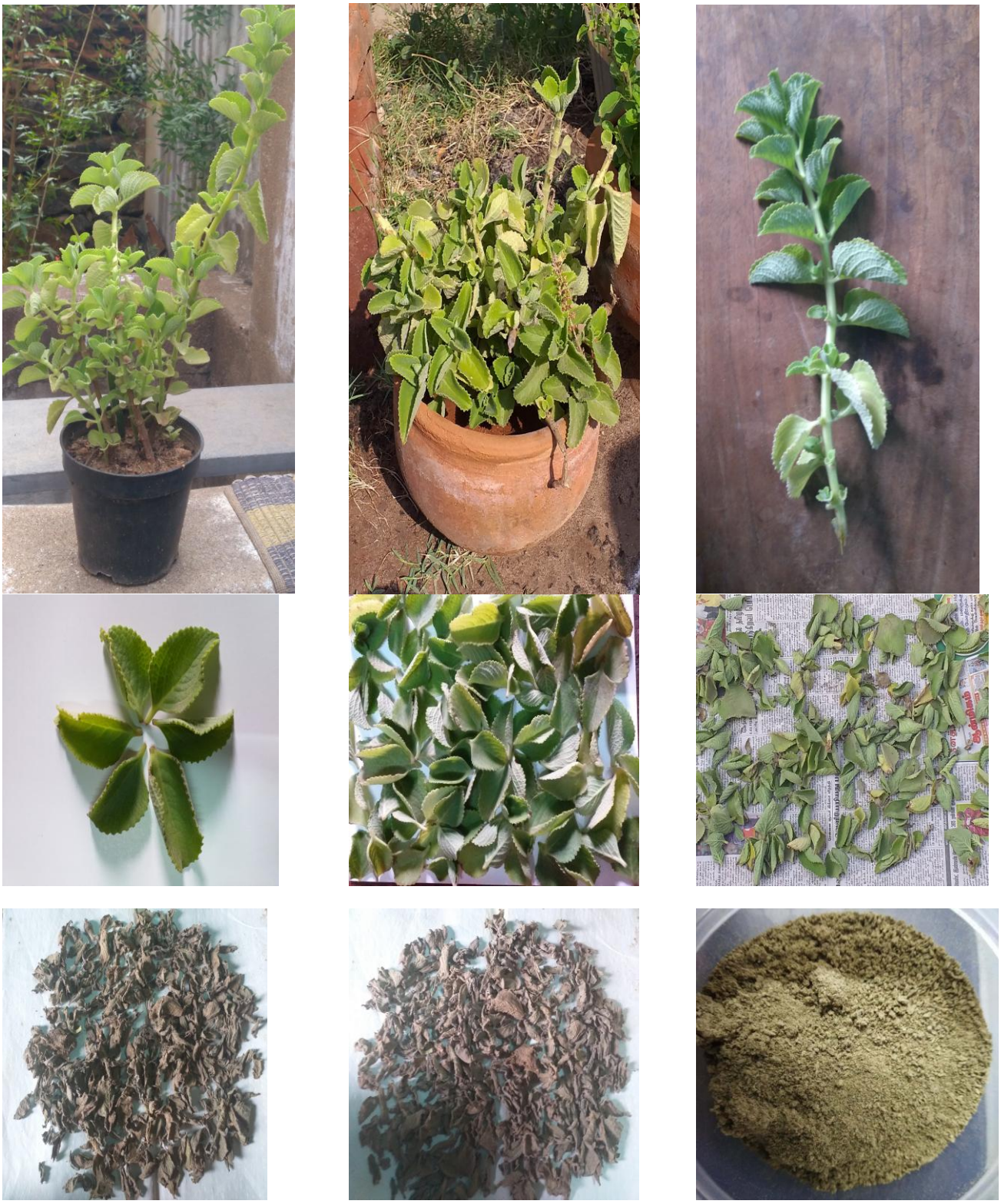


Figure 1. Fine powder of plant sample

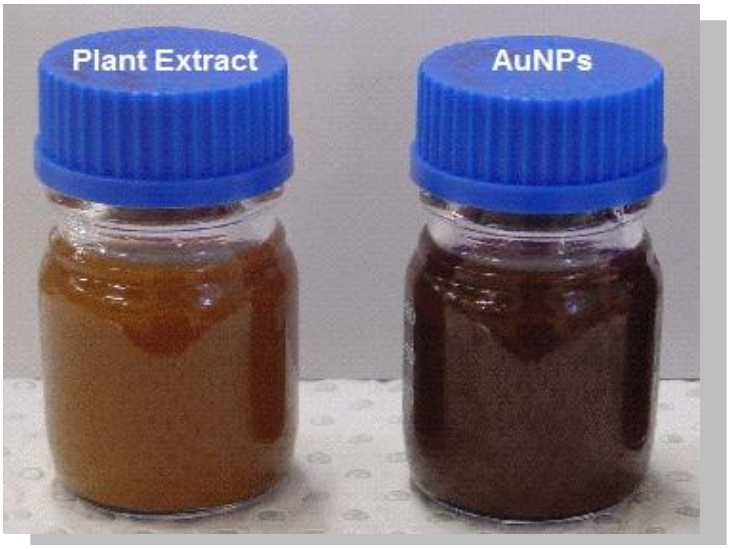


Figure 2. Biosynthesis (Plant mediated) of gold nanoparticles

UV-VIS spectroscopy

Electronic absorption or UV-visible spectroscopy is one of the simplest and yet most useful optical techniques for studying optical and electronic properties of nano materials. This technique is based on the measurement of light absorption by a sample, typically using commercially available spectrometers at reasonable cost. Most spectrometers cover the wavelength range from about 200 nm to 800 nm. The UV-VIS spectroscopic studies revealed the presence of beard peaks at around 540 nm. The Plasmon resonance of the gold nanoparticles was recorded. When the precursor chloroauric acid solutions were mixed with the plant extracts/microbial broth they were reduced into gold (Au) nanoparticles (Figure 3).

The UV-Vis absorption spectroscopy is one of the main techniques followed to examine size and shape of the nanoparticles in the aqueous suspensions (Koperuncholan and Ahmed john 2011). Optical response was recorded under UV-Vis spectroscopy in relation to increase in time duration. The observation of brown and red colors is a characteristic feature for the surface plasmon resonance (SPR) band due to the formation of different sizes of gold nanoparticles in the respective solutions. The transverse plasmon resonance absorption peak appeared at 540 nm is slightly shifted to shorter wavelength along with increase in intensity. The observation of reduction of silver ions present in the aqueous solution of silver complex during reaction with the ingredients of the plant extract may be correlated by the formation of silver nanoparticles in the solution under UV-Vis spectroscopy.

This observation could be attributed to the excitation of surface plasmon vibrations and it has resulted in the formation of silver nanoparticles. Fazal Mohamed et al. (2011) reported that UV-Vis spectrograph of the colloidal solution of silver nanoparticles by changing watery to yellowish brown colour was recorded as a function of time using a quartz cuvette with water as reference in 1:1 ratio of silver ion complex (1 mM) and leaf extract in *Parthenium hysterophorus*. Huang *et al*. (2007) reported formation of silver nanoparticles when constant aqueous $AgNo_3$ at 50 ml, 1 mM with 0.1 g bio-mass produced silver nanoparticles as indicated by sharp absorbance at around 440 nm in *Cinnamomum camphora*.

Koperuncholan and Ahmed john (2011) reported production of gold nanoparticles in Coriander leaf extract at 10^{-3} M aqueous $HAucl_4$ after 12 h due to the excitation of surface plasmon vibrations with the gold nanoparticles as indicated by color change to pink-ruby red. The surface plasmon resonance (SPR) bond of gold occurs initially at about 541 nm after 5 min, increases in intensity as a function of time of reaction and centers at about 550 nm. The plasmon bond of gold nanoparticles is broad with an absorption tail in the longer wavelength that extends well in to the near infrared region attributing to the excitation of in plane surface plasmon resonance and indicates significant anisotropy in the shape of gold nanoparticles.

Fourier Transform Infra-Red (FT-IR) Spectroscopy

The FTIR result of the plant and plant mediated gold nanoparticle are presented in Figure 4 – 5. The FTIR spectrum of the crude leaf extract wherein some pronounced absorbance was recorded in the region between 4000 and 400 cm^{-1}. They include 3432 (secondary amine, free, N-H asymmetric stretching), 2830 (alkyl ethers for C-H stretching), 2085 (isothiocyanates, aromatic N=C=S stretching), 1632 (β-dikeone, enolic form, C=O), 1381 (Alkanes, R-CH_3 symmetric bending), 1353 (Deformation bending for and 652 (C-S, R-C-CH_3 stretching for sulphur compounds), cm^{-1}.

FTIR spectra of the plant extract with gold chloride solution after 5 hrs. such as 3435 (Secondary amine (free) N-H asymmetric stretching), 2829 (Alkyl ethers, C-H stretching), 2728 (Aldehyde, C-H

stretching), 2091 (Isothio-cyanates, Aromatic N=C=S stretching), 1631 (β-diketone (enolic form) C=O), 1353 (Deformation bending, R-C-CH_3) and 644 (Sulphur compounds, C-S stretching) cm^{-1}. In this spectrum, it is found that disappearance of alkanes at 1381 (Alkanes, R-CH_3 symmetric bending) and appearance of C-H stretching of aldehyde at 2728 (Aldehyde, C-H stretching) and the functional groups were as that of the crude extract.

The same solution has polymeric hydroxyl compounds showing O-H stretching at 3400. Aldehyde bond between 2728 and 2730 is present in that solutions at 5 h. Only chloroauric acid ($HAuCl_4$) aqueous solution at 5 h has polychlorinated compounds showing C-Cl stretching. The mechanisms involved in the uptake of metal ions may be intracellular accumulation and surface adsorption. The former one is an active process because the plant must be active to carry out. In the case of surface adsorption, it is a passive process because the chemical groups attached to the cell walls of the plant can bind with metal ions even though when the plant is dead.

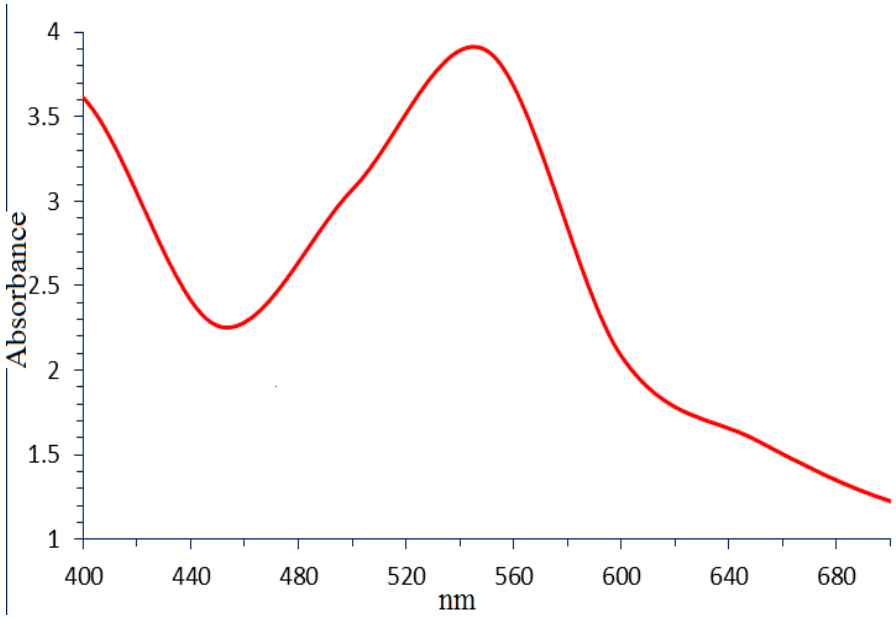
It is considered as an advantage in phytoremediation technologies by which metal contaminants are removed. If the chemical groups attached to the cell walls are the binding sites, then these groups can be adsorbed as metal ions. Therefore, there may be a possibility to use the plant tissues to filter such ions out of the aqueous solutions. This technology is called phytofiltration.

Scanning Electron Microscope (SEM)

SEM absorption of the products was recorded as synthesis of nanoparticles spherical in structure of about 90 nm in diameter in the case of plant derived gold nanoparticles (Figure 6). The energy dispersive spectroscopy is an analysis or chemical characterization of a sample. Leaf extract of *P. amboinicus* was promising one for the development of gold nanoparticles. SEM studies showed spherical-shaped gold nanoparticles at 90 nm in higher densities. Koperuncholan and Ahmed John (2011a) evaluated Alfalfa biomass using SEM micrographs and a corresponding elemental composition of Na, Mg, K, S, Ca, P, Fecdx vacuum (1-270 pa) it allows to observe non-conducting samples without the need to cover them with a thin conductive film, and consequently no evidence of noise by the coating material.

Screening of antimicrobial activity

The antimicrobial activity of test sample was examined with various pathogenic microorganisms using the (measure the inhibition zone) disc diffusion test. The results of the antimicrobial activities are summarized. The two tested concentrations such as 15 and 30 µL /disc produce zone of inhibition on MHA and PDA Figures for bacteria and fungi, respectively. In the present study, higher (30 µL/disc) concentration of sample got greater sensitivity than (15 µL/disc) lower concentration in all the tested microorganisms. Koperuncholan co-workers (2010) stated that the solvent extraction of plant was affected the bacterial strains in the higher concentration such as 2.5 and 5.0 mg/well. But in this study, we conformed that the low concentrations (15 and 30 µL/disc) of the *A. lamarckii* derived AgNPs were highly affect the microbial growth. In this study, all the pathogens were fairly affected and nil effect was not observed in the test samples. In bacteria, the test sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect was noticed from *Micrococcus luteus* NCIM 2871 (B4). In fungi, which was effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect was observed in *Cryptococcus sp.* MTCC 7076 (F2) (Figure 7). All the microbial strains depict higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control except B3, B4 and B6 (Figure 7). There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity was directly related to the sample. The spherical shaped silver nanoparticles having size in range of 16–28 nm were achieved using this extract with antibacterial property observed by Kirby-Bauer method against multi-drug resistant bacteria such as *Streptococcus pyogens, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus* (Vignesh et al. 2012b). A stable and spherical shaped silver nanoparticle was synthesized using extract of *Abutilon indicum*. These nanoparticles show high antimicrobial activities against *S. typhi,E.coli.*



Wavelength	AuNPs
200	3.964
250	3.997
300	3.979
350	3.943
400	3.612
450	2.265
500	3.075
550	3.892
600	2.083
650	1.59
700	1.229
750	1.084
800	1.102

Figure 3. UV-Vis spectrum of plasmon resonance of gold nanoparticles reduced by leaves in *P. amboinicus*

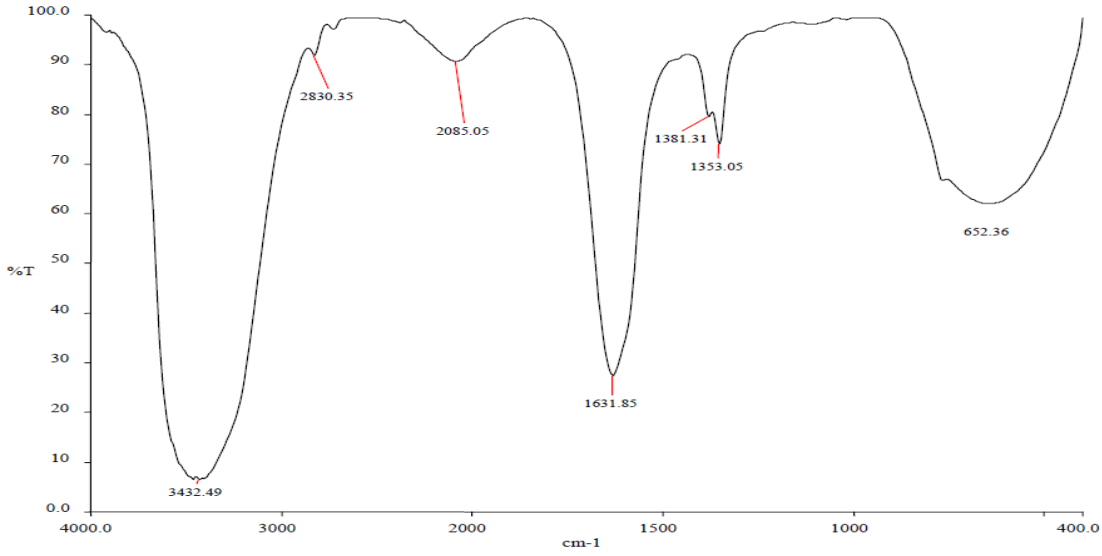


Figure 4. FTIR analysis of vibration modes and function groups of *P. amboinicus* plant extract with gold chloride solution

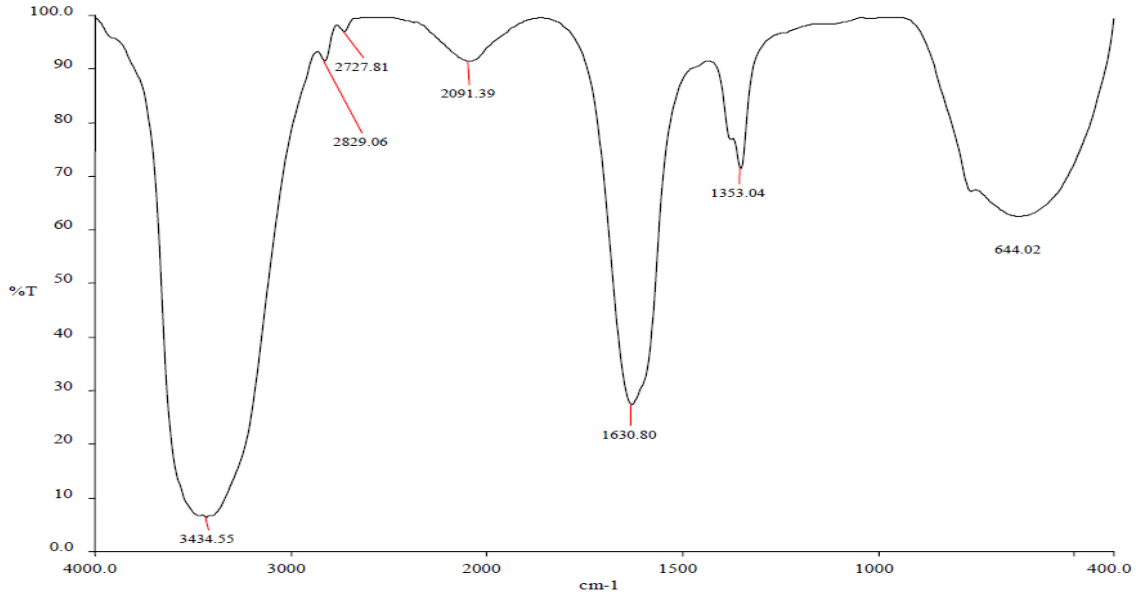


Figure 5. FTIR analysis of vibration modes and function groups of *P. amboinicus*

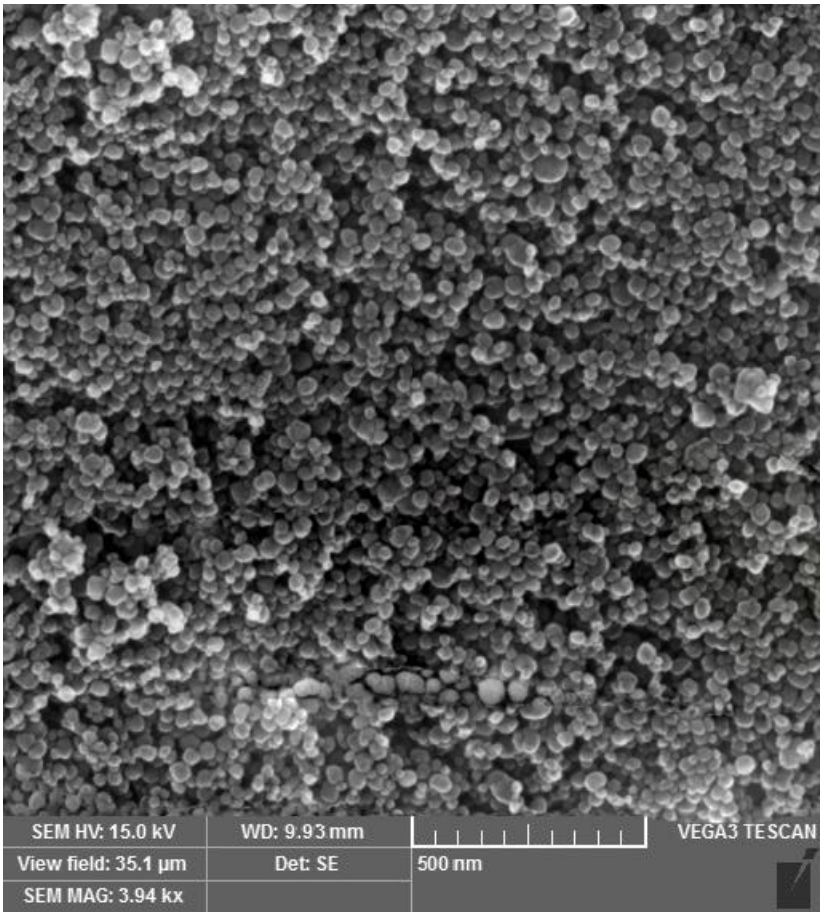


Figure 6. SEM analysis of *P. amboinicus* mediated AuNPs

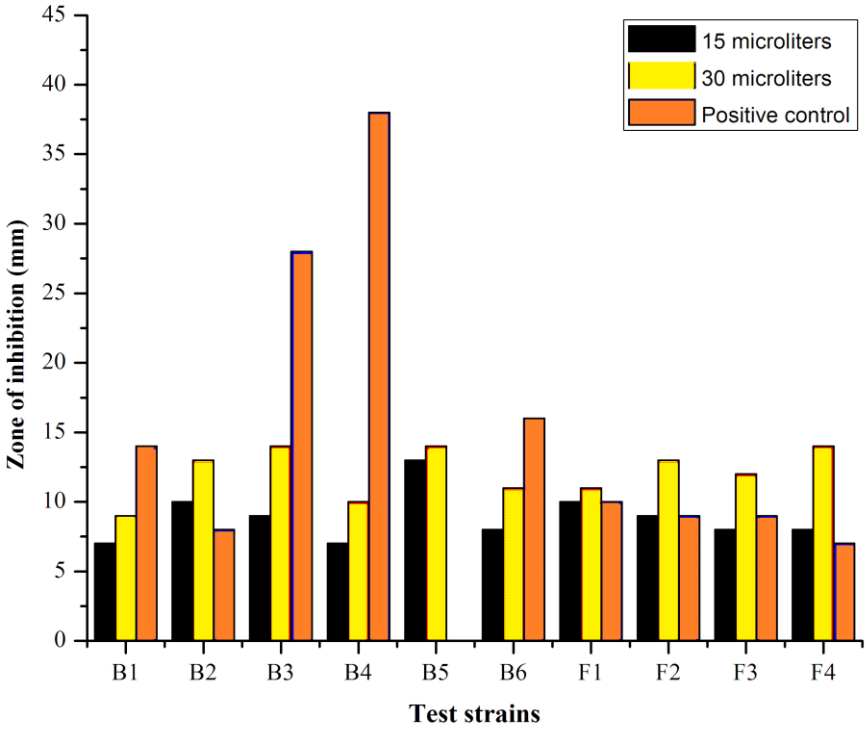


Table 1. *Antimicrobial screening*

S.No	Test Microorganisms	Zone of inhibition (mm) Sample (15 & 30) µL / disc				Diseases	Route of Transmission
Bacteria		**15 µL**	**30 µL**	**PC**	**Remarks**		
1.	*Aeromonas liquefaciens* B1	9	10	14	< PC	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus fecalis* B2	12	13	8	> PC	Endocarditis / Bladder, Prostate	Water / Food
3.	*Klebsiella pneumonia* B3	11	15	28	< *PC*	Acute diarrhea / Dysentery	Water / Food
4.	*Micrococcus luteus* B4	8	10	38	< *PC*	Skin & Pulmonary infections	Soil / Dust / Water
5.	*Salmonella typhimurium*B5	11	12	0	> PC	Typhoid	Water / Food
6.	*Vibrio cholarae* B6	9	12	16	< *PC*	Cholera	Water / Food
Fungi							
7.	*Candida albicans* F1	11	12	10	> PC	Skin (Integument) Infections /	Airways / Wound /
8.	*Cryptococcus* sp. F2	10	12	9	> PC	Cryptococcal disease /	Airways / Wound /
9.	*Microsporum canis F3*	10	11	9	> PC	Tinea capitis /Ringworm	Airways / Wound /
10.	*Trichophyton rubrum* F4	11	13	7	> PC	Tinea corporis / Tinea cruris / Tinea	Airways / Wound /

Here, i am giving the mean value of the result (3 replicates)
PC - Positive Control (Using antibiotic disc; **Bacteria – Methicillin (10mcg/disc) ; Fungi – Itraconazole (10mcg/disc)**
Samples - 15 µL / disc & 30 µL / disc; **> PC – greater than positive control; < PC – less than positive control**

Conclusion

In our present investigation, we conducted an in-depth study on the synthesis and characterization of Au nanoparticles and their application on biological system. Antibacterial and Antifungal efficacy of the Au nanoparticles against six different bacteria and four different fungus has been performed. At nanoscale, gold exhibits remarkably unusual physical, chemical and biological properties. Effective green synthesis of nanoparticles will have greater implication and application in biomedical research. The mechanisms involved in the uptake of metal ions may be intracellular accumulation and surface adsorption. In this study nanoparticles of 70 ± 80 nm were synthesized by using *P. amboinicus*, as confirmed by SEM and EDAX. These nanoparticles showed characteristic absorption peak at 540 nm in UV spectra. The possibility of protein as a stabilizing material in gold nanoparticles is revealed by FTIR analysis. The crystalline structure of gold nanoparticles was confirmed by XRD. The antimicrobial study was confirmed that Au biosynthesized nanoparticles will act as an alternative antibiotic in future.

Acknowledgments

The authors thank the Biospark Biotechnological Research Center (BBRC), Tiruchirappalli, Tamil Nadu, India for XRD and SEM/EDS analysis.

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