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GOLD NANO DRUG DESIGN FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT: A bioreductive approach to the synthesis of gold nanoparticles in the leaves of *Asystasia gangetica* demonstrates the formation of spherical gold nanoparticles and triangular, rod, oval-and circle-shaped gold nanoparticles to the aqueous solutions in respective ranges. The marked difference in the shape of the gold nanoparticles has attributed to the comparative advantage of protective biomolecules and reductive biomolecules. The results were verified using UV-Vis spectroscopy, FTIR and SEM. The AuNPs were monodisperse and found to be 10-100 nm in size. The AuNPs had challenged against certain pathogenic bacterial and fungal strains. In antimicrobial activity, the AuNPs has most effective against *Salmonella typhimurium* while smaller effect has noticed from *Micrococcus luteus*.

KEYWORDS: *Asystasia gangetica*, Antimicrobial activity, Gold Nanoparticles.

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1.INTRODUCTION

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic Agents [1]. There are some new approaches to drug discovery, such as combinatorial chemistry, nanotechnology and computer-based molecular modelling design, none of them can replaced the important role of natural products in drug discovery and development [2]. *Asystasia gangetica* (Chinese violet) Perennial herbs, erect, decumbent, or clambering; 1-3 m in length. Leaves opposite; blades simple, with numerous linear cystoliths on the upper surface, the

secondary veins conspicuous; stipules absent. Species is native to tropical Malaysia, India, and Africa, but it has introduced into tropical areas in North, Central and South America, Hawaii, West Indies, and Australia as an ornamental herb and eventually escapes into natural and disturbed areas. The family Acanthaceae includes about 4000 species widespread in both New and Old World Tropics. This family includes a range of morphological diversity, habitats, and biogeographic patterns. The genus Asystasia includes about 40 species of paleotropical origin [3], [4], and [5]. Nanotechnology is the science of the extremely tiny and involves the study and use of materials on an unimaginable small scale [6], [7]. Currently, there is a growing need to develop environmentally benign nanoparticle synthesis process that does not use toxic chemicals in the synthesis protocols. Gold nanoparticles have gained increasing interest due to their specific features [8], [9]. Such as unusual optical and electronic properties, noncytotoxicity [10], [11]. high stability, biological compatibility, controllable morphology, size dispersion and easy surface functionalization. In the present investigation, the plant material *A. gangetica* a traditional medicine well known for its curative properties and it has been used to biosynthesis of gold nanoparticles characterization for their antimicrobial activity.

2. MATERIALS AND METHODS

Study area and sampling

The plant *A. gangetica* has collected from Tiruchirappalli District of Tamil Nadu in India during the period of June to July 2018.

Preparation of Leaf Extracts

The leaves has collected individually from the plant, washed thoroughly thrice with distilled water; shade dried up to 5 days and ground into fine powder. The fine powder of the plant material has sterilized at 121 °C for 15 min and weighed. Sterilized fine powder, 20 g each has taken, mixed with 200 ml of Milli-Q water and kept in boiling water bath at 60 °C for 10 min. The extracts has filtered with Whatman 1 filter paper and the filtered extracts were stored in a refrigerator at 4°C for further studies to avoid microbial contamination.

Biofabrication of nanoparticles

The aqueous solution of gold chloride (1 mM) was mixed with above said filtrate and the flasks were agitated at 37 ^{0}C. The saline bottles has tightly covered with aluminum foil in order to avoid photo reduction of gold ions, incubated at room temperature under dark condition and observations has recorded. Periodically, Aliquots of only those isolates which showed colour change from white to purple were subjected to UV–visible absorption spectrophotometry, Fourier Transform Infra-Red (FT-IR) Spectroscopy and SEM studies. Control without gold chloride has also run along with the experimental flasks.

Characterization of gold nanoparticles

UV-VIS Spectroscopy

A small quantity of biosynthesized nanoparticles has characterized by UV-VIS spectroscopy. After colour development, a small aliquot of the solution was absorbed between 200 and 900 nm under UV-VIS spectroscopy.

Fourier Transform-Infra Red (FT-IR) Spectroscopy

The analysis of bio-reducing agent present in each of the extracts has measured by FTIR. After the reaction, a small aliquot of the concentrated reaction mixture has measured in the transmittance mode at 400 to 4000 cm^{-1}. The spectra of the extracts taken before and after the biosynthesis of nanoparticles has analysed [12].

Scanning Electron Microscopy (SEM)

The aqueous solution containing gold nanoparticles has subjected to cooling centrifugation at 6000 rpm for 10 min. Supernatant solution was decanted and the remains present as thin-layer solid material was collected, dried in hot air oven at 60°C until complete drying and examined under scanning electron microscopy (MODEL JEOL, JSM-5610) at different magnifications (10,000 X and 40,000 X).

Assay of antimicrobial activity

Microbial strains: *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). Tested for antimicrobial sensitivity using the disc diffusion method [12], [13]. The cultures has obtained from MTCC, Chandigarh and NCIM, Pune, India. This method has used to evaluate in vitro antibacterial and antifungal activity of test sample against certain human pathogenic microorganisms on Muller Hinton Agar (MHA) and Potato Dextrose Agar (PDA), respectively [14], [15]. A sterile cotton swab has used to inoculate the standardized bacterial suspension (test culture suspensions prepared in sterile 0.85% saline matching an optical density of 0.5 McFarland standards corresponding to 10^8 CFU/ml) on surface of agar for rotating the every 60° to ensure homogeneous growth [16],[17]. (Vignesh et al., 2015a; 2015b). The 15 and 30 µL of test solutions has poured in each disc, separately. One separate disc has used for control study by taking sterile triple distilled water (without test sample). Incubated at 37±1°C for 24–48 h (for bacteria) and 25±1°C for 48-72 h (for fungus). After incubation, the zone of inhibition has measured with ruler/HiAntibiotic ZoneScale-C [18], [19]. The assays had performed in triplicate and the average values have presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) has used as positive control. All the media, standard discs and HiAntibiotic ZoneScale-C has purchased from Hi-Media (Mumbai, India) [20].

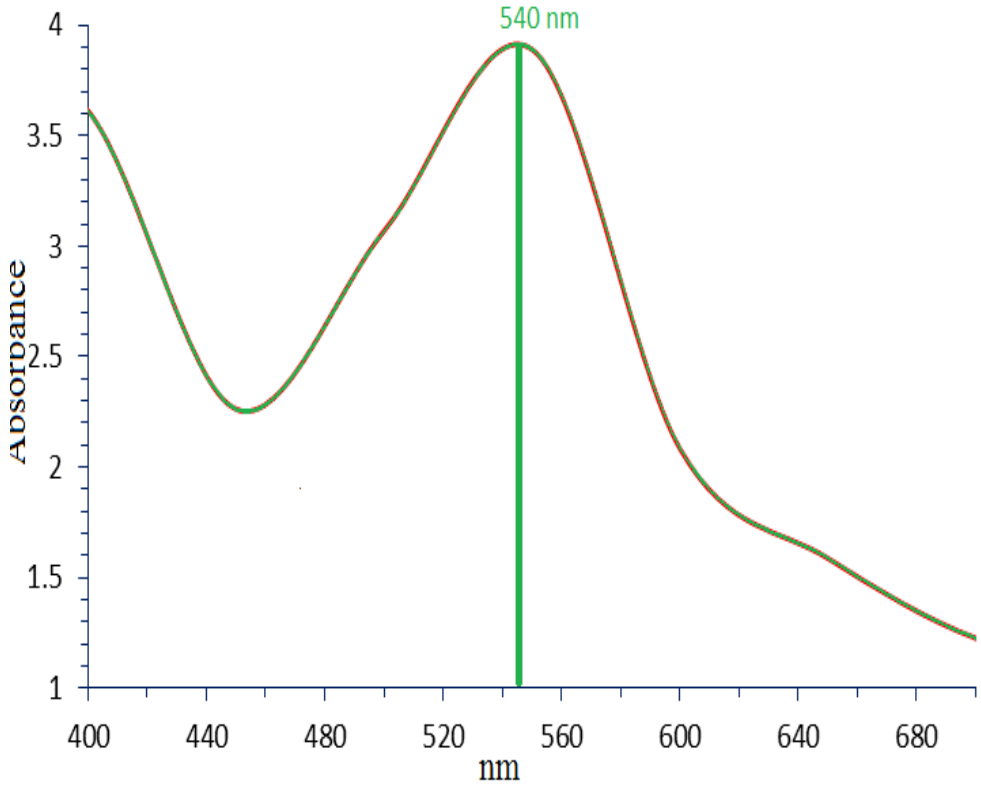
3. RESULTS AND DISCUSSION

Biofabrication of Au nanoparticles

When the *A. gangetica* leaves fine powder aqueous extract has mixed at 0.1% concentration of chloroauric acid ($HAuCl_4$) aqueous solutions the solutions changed their colour from pale brown to pink for gold nanoparticles. The change in colour is due to the excitation of surface plasmon vibration, which has indicated by the formation of gold nanoparticles at different time intervals. Spectroscopic data had analysed to characterize gold nanoparticles.

UV-VIS spectral analysis

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 has 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 has recorded. When the precursor chloroauric acid solutions has mixed with the plant extracts /microbial broth, they has reduced into gold (Au) nanoparticles (Figure 1). The UV-Vis absorption spectroscopy is one of the main techniques followed to examine size and shape of the nanoparticles in the aqueous suspensions [21], [22]. Optical response has recorded under UV-Vis spectroscopy in relation to increase in time duration. The observation of brown and red colours 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 correlated by the formation of silver nanoparticles in the solution under UV-Vis spectroscopy.



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 1. UV-Vis spectrum of plasmon resonance of gold nanoparticles reduced by leaves in *A. gangetica*

Fourier transform infra-red (FTIR) spectroscopy

The FTIR result of the plant and plant mediated gold nanoparticle has presented in Figure 2 and 3. The FTIR spectrum of the crude leaf extract wherein some pronounced absorbance has 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 solution 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 [23]. It has considered as an advantage in phytoremediation technologies by which metal contaminants had removed. If the chemical groups attached to the cell walls are the binding sites, then these groups has 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 called phytofiltration.

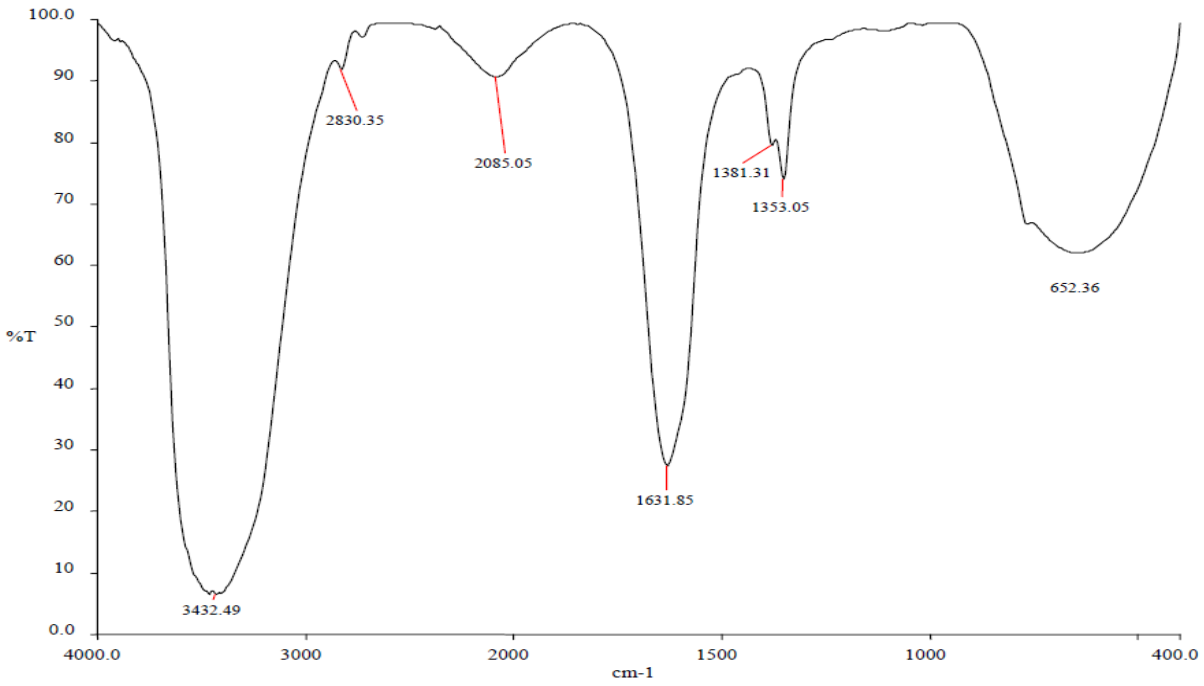


Figure 2. FTIR analysis of vibration modes and function groups of *A. gangetica* plant extract with gold chloride solution

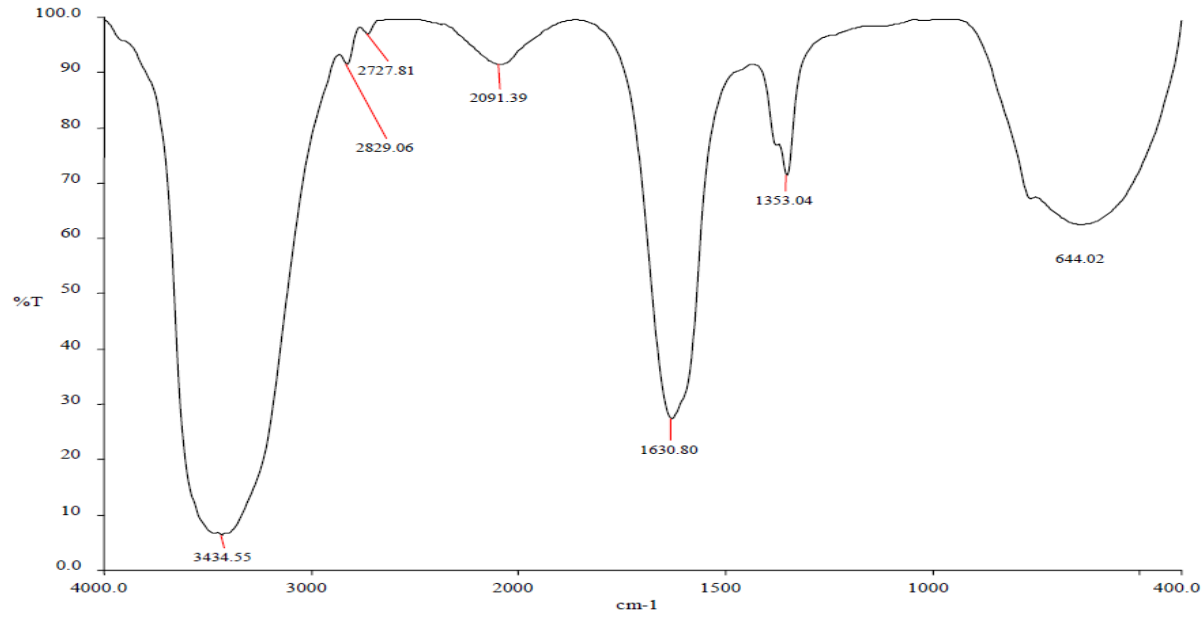


Figure 3. FTIR analysis of vibration modes and function groups of *A. gangetica*

Scanning electron microscope (SEM)

SEM absorption of the products has recorded as synthesis of nanoparticles spherical in structure of about 90 nm in diameter in the case of plant derived gold nanoparticles (Figure 4). SEM studies showed spherical-shaped gold nanoparticles at 90 nm in higher densities [24]. 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.

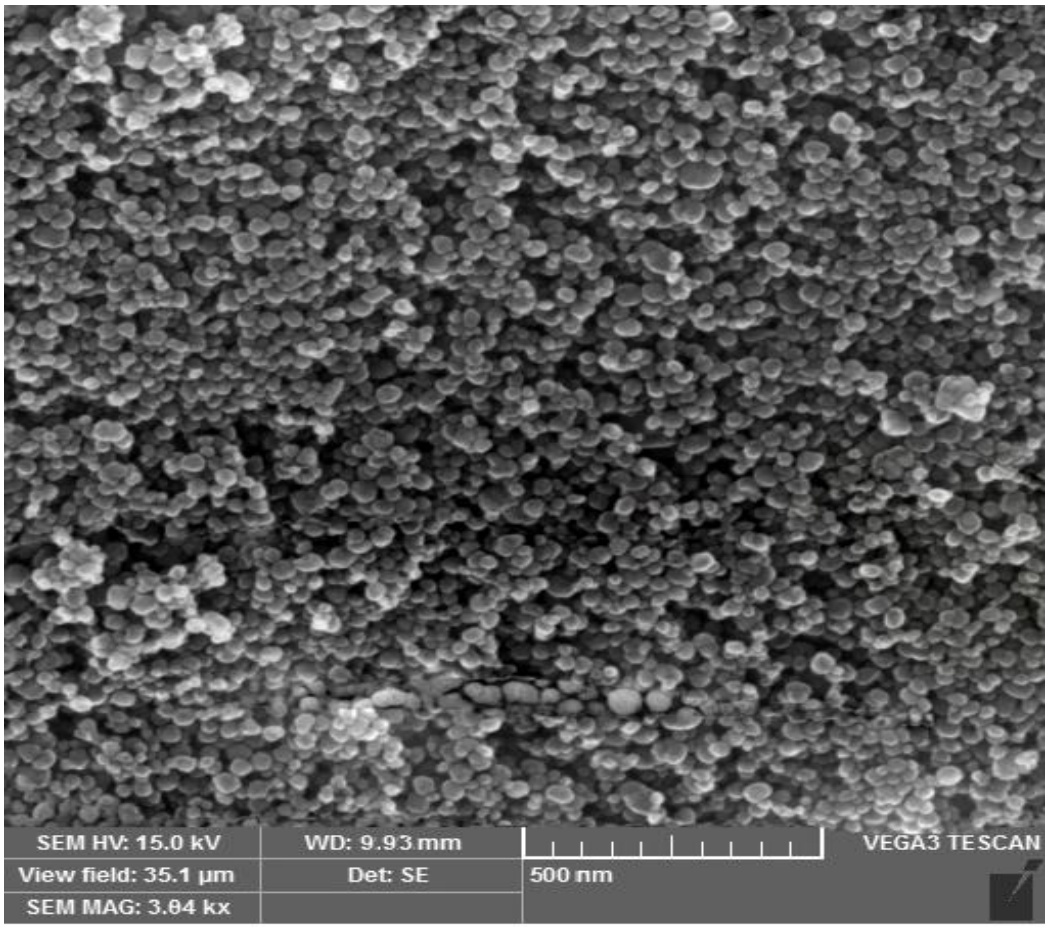


Figure 4. SEM analysis of *A. gangetica* mediated AuNPs

Antibacterial and antifungal screening

The antimicrobial activity of test sample has examined with various pathogenic microorganisms using the (measure the inhibition zone) disc diffusion test. The results of the antimicrobial activities has 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 has fairly affected and nil effect has not observed in the test samples. In bacteria, the test sample was most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect has 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). 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 (Table.1 and Chart. 1). 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 [25], [26], [27]. 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* [28], [29]. 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, S. aureus* and B. *substilus* microorganisms [30], [31].

Table 1: Antimicrobial screening of AuNPs

S.No	Test Microorganisms	Zone of inhibition (mm)				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	14	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	12	38	< *PC*	Skin & Pulmonary infections	Soil / Dust / Water
5.	*Salmonella typhimurium* B5	13	15	0	> PC	Typhoid	Water / Food
6.	*Vibrio cholarae* B6	8	12	16	< *PC*	Cholera	Water / Food
Fungi							
7.	*Candida albicans* F1	11	12	10	> PC	Skin (Integument) Infections	Airways / Wound
8.	*Cryptococcus* sp. F2	9	11	9	> PC	Cryptococcal disease /	Airways / Wound

9.	*Microsporum canis* F3	10	12	9	> PC	Tinea capitis /Ringworm	Airways / Wound
10.	*Trichophyton rubrum* F4	10	11	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

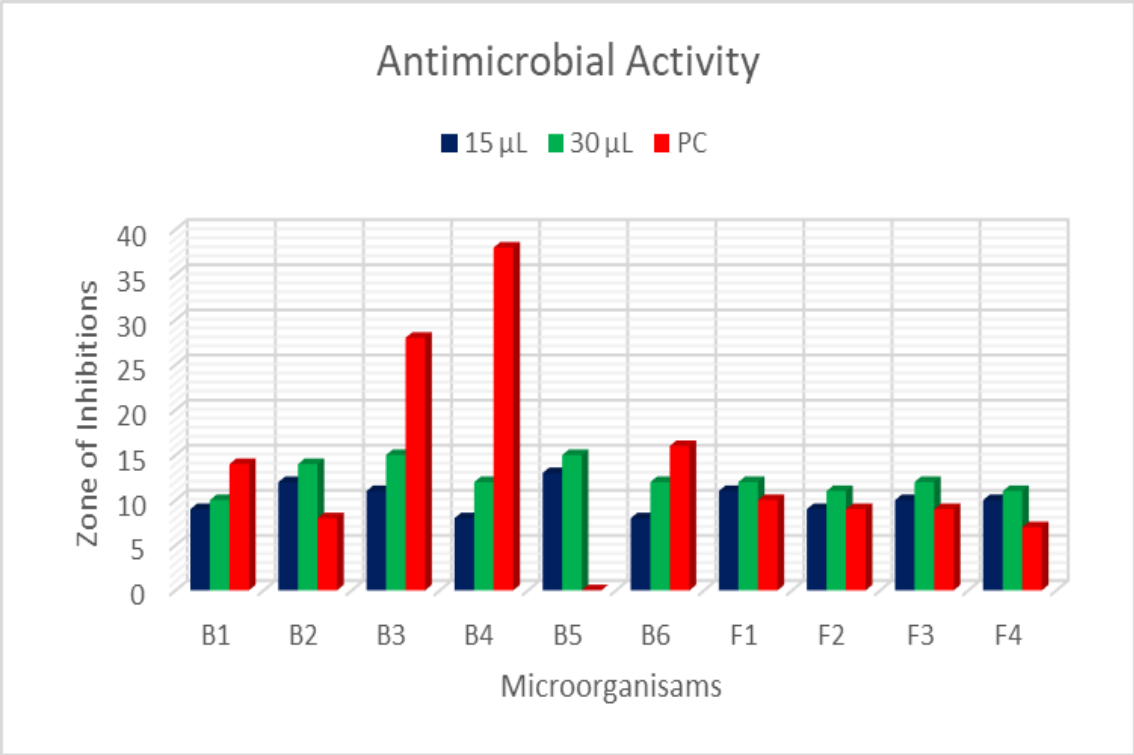


Chart .1 Antimicrobial Activity of gold nanoparticle

4. CONCLUSION

Instead of the boiled leaf broth method followed in the previous studies, leaves of *A. gangetica* appear to be environmental-friendly and low-cost candidate as a reductant for synthesizing gold nanoparticles. This procedure has extended to the synthesis of other nanoparticles from different chemical compositions. Synthesis of nanoparticles has many advantages by scale up of each process because of its economic viability, possibility of covering large surface area easily by suitable growth of the filaments, etc. Equally, the synthesis of metal ion reduction or reaction process in cellular metabolism explaining whether the nanoparticles formed as by-products of the process has role to play in a cellular activity. In this low cost procedure, effective synthesis of nanoparticles will have greater implication and application in biomedical research.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

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