

SYNTHESIS OF GOLD NANOPARTICLES USING *XANTHIUM STRUMARIUM* LEAVES EXTRACT AND THEIR ANTIMICROBIAL STUDIES: A GREEN APPROACH

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ABSTRACT

A simple one-pot green approach for the biosynthesis of gold nanoparticles (AuNPs) by reducing chloroauric acid (HAuCl₄) with extract of *Xanthium strumarium* or *Xanthium Indicum Kone* is described. The biosynthesized new materials were extensively characterized by UV Visible spectroscopy, FT-IR, XRD, SEM and TEM analysis. The formation of Gold nanoparticles from plant material was confirmed. TEM image revealed that most of the particles were found in a spherical shape with size ranging from 9.60 nm to 11.70 nm. The size, shape and purity were well analyzed. The biological profile such as *In vitro* antibacterial activity and *In vitro* antifungal activity was investigated and shows the significance of their application towards biological activity.

Keywords: Nanoparticles, biological activity, *Xanthium Indicum Kone*, AuNPs, Green Synthesis

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INTRODUCTION

In recent years, nanotechnology combined with other branches of science is the main growing area of research.¹⁻³ Development of new biomaterials using metal and nonmetal nanoparticles has been extensively reviewed in the literature.⁴⁻⁷ More specifically, the development of biosynthesized nanoparticles and their utility, applications is one of the important research in the concern of environmental chemistry. Green Synthesis, which provides benefit over conventional method as it is cost-effective, eco-friendly, easily scaled up for large-scale synthesis and the present method is no need of high pressure, energy, temperature and toxic chemicals.⁶ Green approach for the synthesis of nanoparticles⁸⁻¹⁰ is a major objective of research in recent years because of less usage of hazardous chemicals. Interestingly, in the point of nanotechnology concerned with the development of immediate and very easy methods of experimental protocols for the synthesis of green metal-nanomaterials which includes a wide range of chemical compositions and different size. In the continuation of our research interest on the green synthesis of nanoparticles using various plants and their materials, we present our report in this manuscript on the synthesis of gold nanoparticles using *Xanthium strumarium* Leaf extract.

Xanthium strumarium or *Xanthium Indicum J. Koenig ex Roxb.* is a species of annual plants belong to the *Asteraceae* family and commonly known as "Cocklebur".¹¹⁻¹² It may originate in North America and has been extensively spread out elsewhere. However, Small quantities of this plant may be consumed, but seeds of this plant should not be eaten in large quantities because of their toxic chemical carboxyatratyloside. The plant's root and fruit, is used as traditional medicine. It is used for the diseases leucoderma, biliousness, poisonous bites of insects, epilepsy, salivation and fever. This plant's leaf material has been found to have strong antimicrobial activity and there is no toxic. This plant has various useful biological activities and found in literature.¹¹⁻¹² However, metal capped nano-size material with this plant is not yet known. To the best of our knowledge, this is the first report on gold nanoparticle using *Xanthium*

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strumarium Leaf extract. In this paper, we reported our recent research on green approach for the synthesis of *Xanthium strumarium* Leaf extract mediated gold nanoparticle, their characterization and Antibacterial, Antifungal Studies. The protocol used in this work is very simple, greener, eco-friendly, and no other capping agent required.

EXPERIMENTAL

Sample Collection

The fresh leaves of *Xanthium strumarium* were collected from palayamkottai in Tirunelveli district of Tamil Nadu, India.

Preparation of *Xanthium Strumarium* Leaf Extract

The fresh leaves of *Xanthium strumarium* were individually collected and washed thoroughly with cold water to remove any debris and dust attached to the leaves and then with distilled water, up to 10 days shade dried and fine powder was prepared by using a mechanical grinder. 5 gram of the fine powder was mixed with 200 ml de-ionized water and boiled for 10 minutes at 50 °C using a water bath. The extract was filtered with Whatmann filter No.1 paper to get the clear aqueous extract. The filtrate was used for the synthesis of gold nanoparticles.

Biosynthesis of Gold Nanoparticles

Chloroauric acid (HAuCl_4) was used as received. 90 mL of 1mM solution of chloroauric acid and 10 mL of *Xanthium strumarium* leaf extract was added drop wisely. The color change is observed from pale yellow to red in color. The reaction mixture is stirred for 5 minutes at room temperature. This color change clearly indicates that the reduction of gold (Au^+) to Au nanoparticles by the plant materials. The formed materials were used for further investigations. The formed precipitate washed with water several times to remove if any impurities present. Finally, we obtained dark brown color solid mass which is dried in hot air oven at 60 °C for 24 h. The fully dried dark brown colored powder was kept in air tight container and used for further analysis.

RESULTS AND DISCUSSION

The synthesis of gold nanoparticles using *Xanthium strumarium* leaf extract was accomplished by the aforementioned procedure. The benefits of the synthesis are i) it can be performed in minutes rather than hours, (ii) under very mild conditions, (iii) without using any capping agent. We observed the color change from yellow to red and it indicates the formation of gold nanoparticles via reduction of gold by aqueous plant extract. The formation of gold nanoparticle is confirmed by UV-Vis absorption spectrum and it showed the surface plasmon band at around 553 nm and it showed in Fig.-1. FTIR analysis showed that reduction made by carboxyl and alcoholic groups those are present in the leaves of plant extract. The comparison of FT-IR spectra clearly shows that the nanoparticle binds with a natural product (Characteristic bands of carboxylic and alcoholic groups, See Fig.-2).

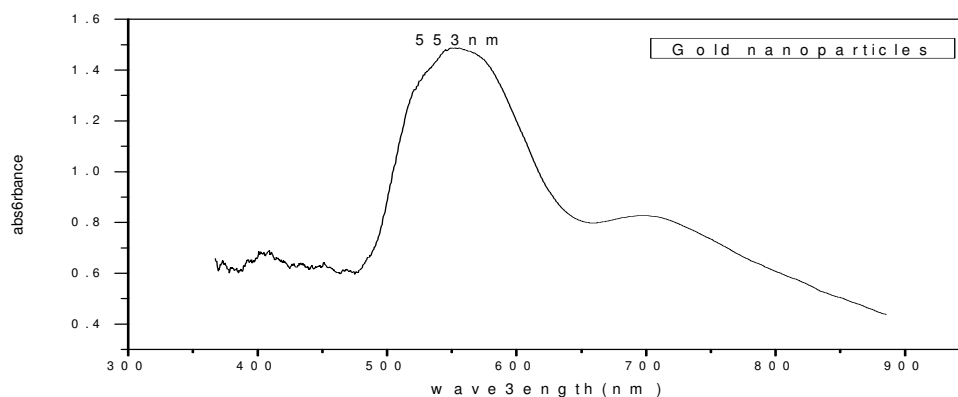


Fig.-1: UV Spectrum of Gold Nanoparticle using *Xanthium strumarium* Leaf Extract

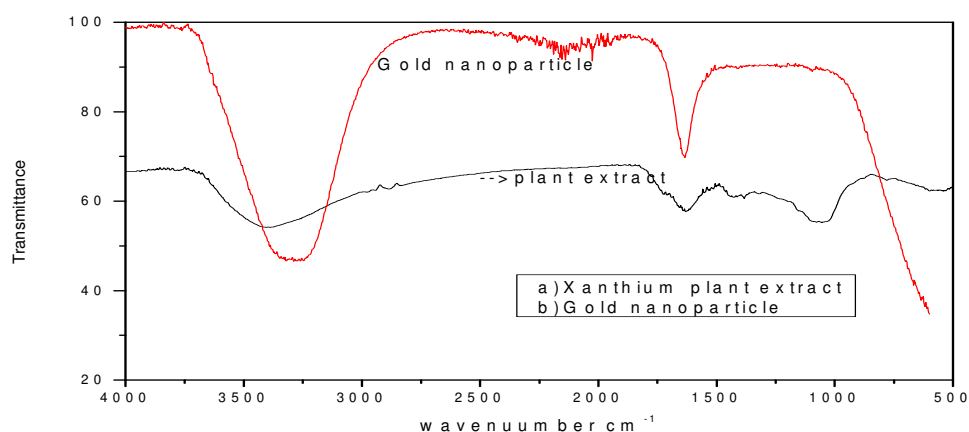


Fig.-2: IR Analysis of Gold Nanoparticle and *Xanthium strumarium* Leaf Extract

The presence of gold and its oxidation state was confirmed by powder X-ray diffraction pattern. Two theta values around 38 and 44 clearly indicate the presence of gold in zero oxidation state and shown in Fig.-3.

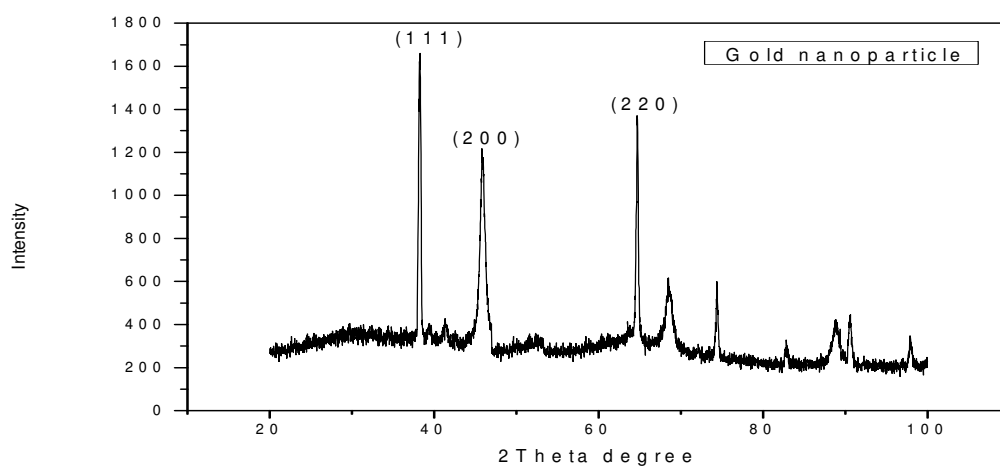
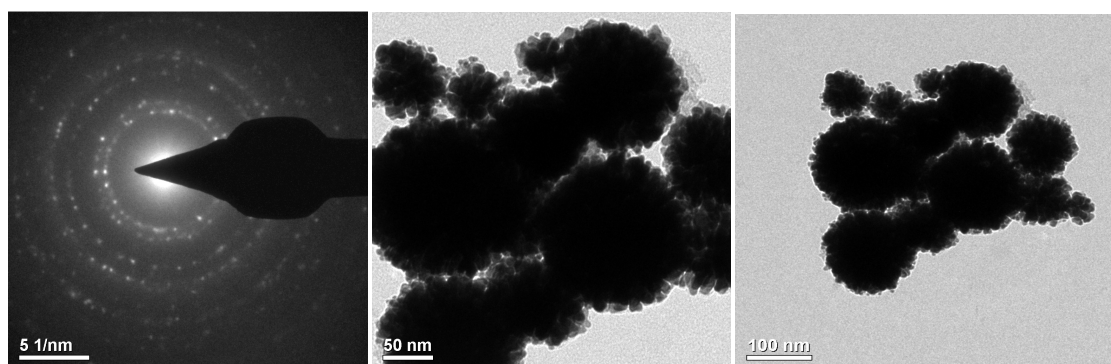
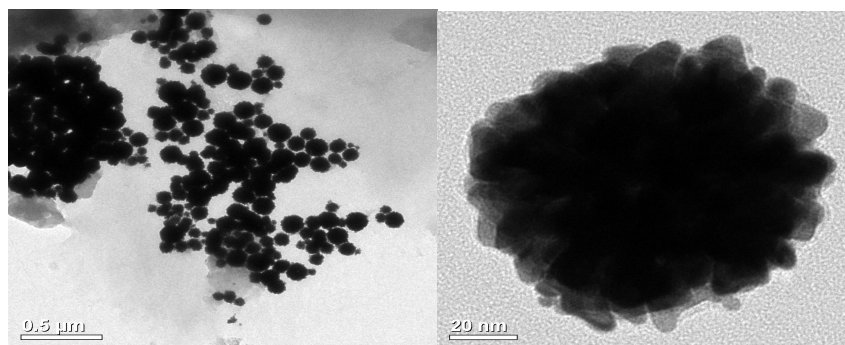
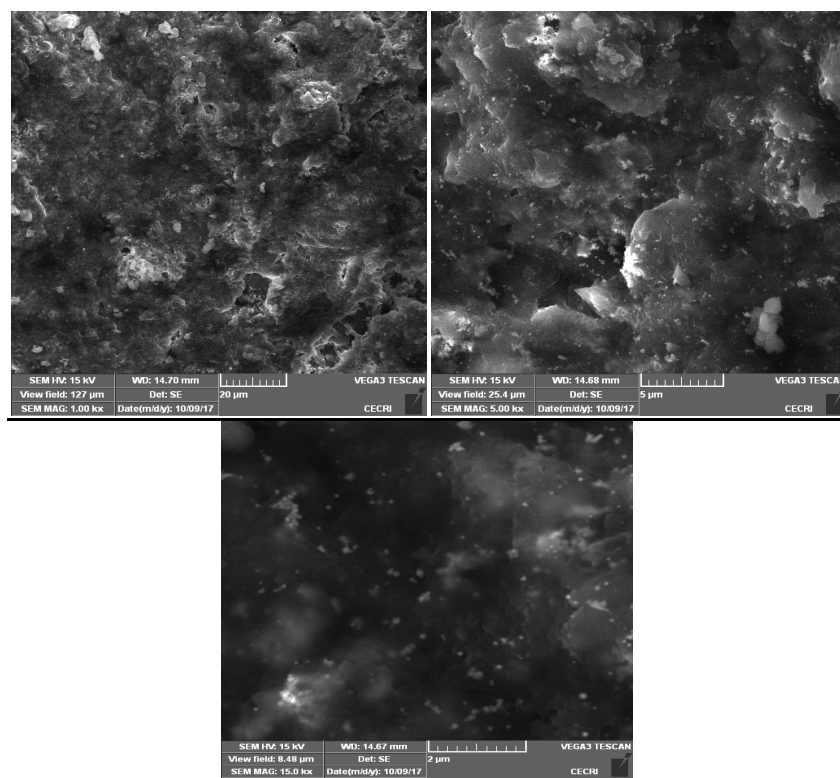
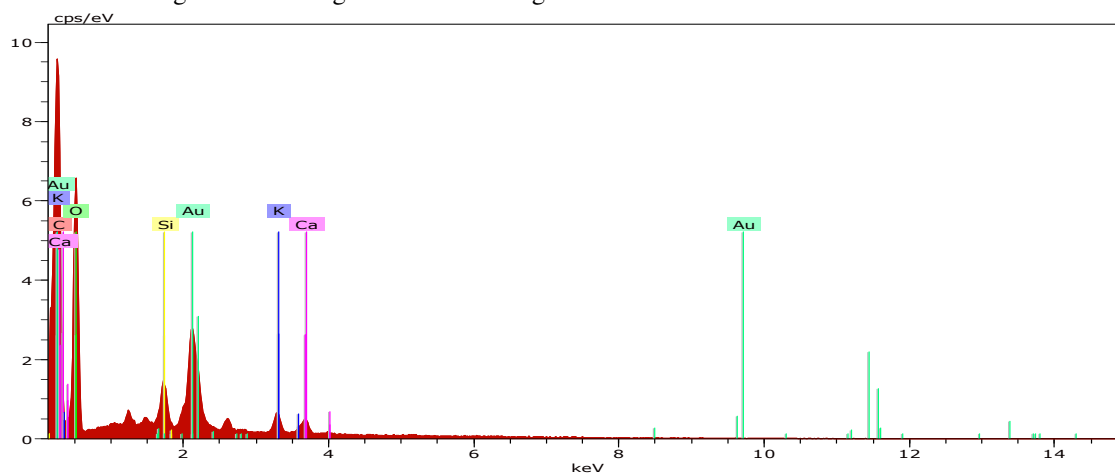


Fig.-3: Powder XRD Pattern of Gold Nanoparticle using *Xanthium strumarium* Leaf Extract

Analysis of SEM and TEM clearly indicate the shape of the particles (Fig.-4 and Fig.-5). TEM image clearly revealed that nanoparticles were found in a spherical shape with size ranging from 9.60 nm to 11.70 nm (Fig.-4). The EDX analysis shows the presence of gold, oxygen, carbon. The results clearly indicating that plant material is fabricated with gold. EDX analysis is shown in Fig.-6.



Fig.-4: TEM Images of Gold Nanoparticle using *Xanthium strumarium* Leaf ExtractFig.-5: SEM Images of AuNPs using *Xanthium strumarium* Leaves ExtractFig.-6: EDX Analysis of AuNPs using *Xanthium strumarium* Leaves Extract

Anti-Microbial Studies

The antibacterial activity was determined by well diffusion methods.¹² Pathogenic bacteria, such as *Staphylococcus aureus* (MTCC 96) and *Pseudomonas aeruginosa* (MTCC 2453) *Escherichia coli* (MTCC 443), *Klebsiella pneumonia* (MTCC 530), and *Bacillus subtilis* (MTCC 441) were used for *in vitro* antimicrobial activity. These selected pathogenic strains were purchased from Microbial Type Culture Collection, IMTECH, Chandigarh. The results are shown in Table-1, Fig.-7 and Fig.-8.

The antifungal activity of *Xanthium strumarium* NPs was determined by well diffusion methods.¹¹ Five fungal strains such as, *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 277), *Aspergillus terreus* (MTCC 1782) *Fusarium oxysporum* (MTCC 284) and *Candida albicans* (MTCC 227) were used for *in vitro* antifungal activity. The *Xanthium strumarium* NPs of sample 1 was effectively inhibited the all test pathogens at higher tested concentrations. The lowest concentration of 50 µg/well did not show the inhibition. The results are shown in Table-2 and Fig.-9 and 10.

Antibacterial Studies

The antibacterial activity of *Xanthium strumarium* NPs was determined by well diffusion methods.¹³⁻¹⁵ About 25 mL of molten Mueller Hinton agar was poured into a sterile Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which 18 h grown (OD adjusted to 0.6) 100 µl of aforesaid pathogenic bacteria were transferred onto a plate and made culture lawn by using a sterile L-rod spreader. After 5 minutes setting of the pathogenic microbes, a sterile cork borer was used to make 5 mm well on the agar. The test samples were dissolved in sterile saline and then loaded into wells with various concentrations such as 25 µg/well, 50 µg/well, 75 µg/well and 100 µg/well. The solvent saline loaded well served as negative control and Azithromycin (30µg/ml) well served as positive control. The samples were incubated at 37 °C in a 40 W florescent light source (~ 400 nm) for 24 h. The antibacterial activity *Xanthium strumarium* NPs were determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale. Figure-7 and 8 show the antibacterial study of *Xanthium strumarium* NPs.

Table-1: Antibacterial activity of *Xanthium strumarium* NPs

S. No.	Name of organisms	Zone of inhibition(mm)				
		25	50	75	100	Azithromycin (30)
1	<i>Staphylococcus aureus</i>	8	10	14	18	24
2	<i>Escherichia coli</i>	6	12	16	19	23
3	<i>Pseudomonas aeruginosa</i>	9	11	17	20	22
4	<i>Klebsiella pneumoniae</i>	7	12	13	16	20
5	<i>Bacillus subtilis</i>	5	9	12	16	26

Antifungal Studies

Five fungal strains such as, *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 277), *Aspergillus terreus* (MTCC 1782) *Fusarium oxysporum* (MTCC 284) and *Candida albicans* (MTCC 227) were used for *in vitro* antifungal activity. These selected strains were obtained from MTCC.

In vitro Antifungal Activity

The antifungal activity of *Xanthium strumarium* NPs was determined by well diffusion methods.¹³⁻¹⁵ About 25 mL of potato dextrose agar was poured into a sterile Petri dish. The plates were allowed to solidify, after which two days grew fungal disc (5 mm) of mycelial fungal pathogens were placed separately on to the mid of the agar plate and wells were made. For yeast fungi, 18 h grown culture was set to 0.6 OD and swabbed using a sterile cotton swab. The test sample was dissolved in sterile water and loaded into wells with various concentrations such as 25 µg/well, 50 µg/well, 75 µg/well, 100 µg/well. The clotrimazole added well served as positive control. All the drug-loaded plates were kept for 72 h. The antifungal activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale. The test material was effectively inhibited the all test pathogens at higher tested concentrations. The lowest concentration of 50 µg/well did not show the inhibition. The results spectrum of the tested drug was given in the Table-2.

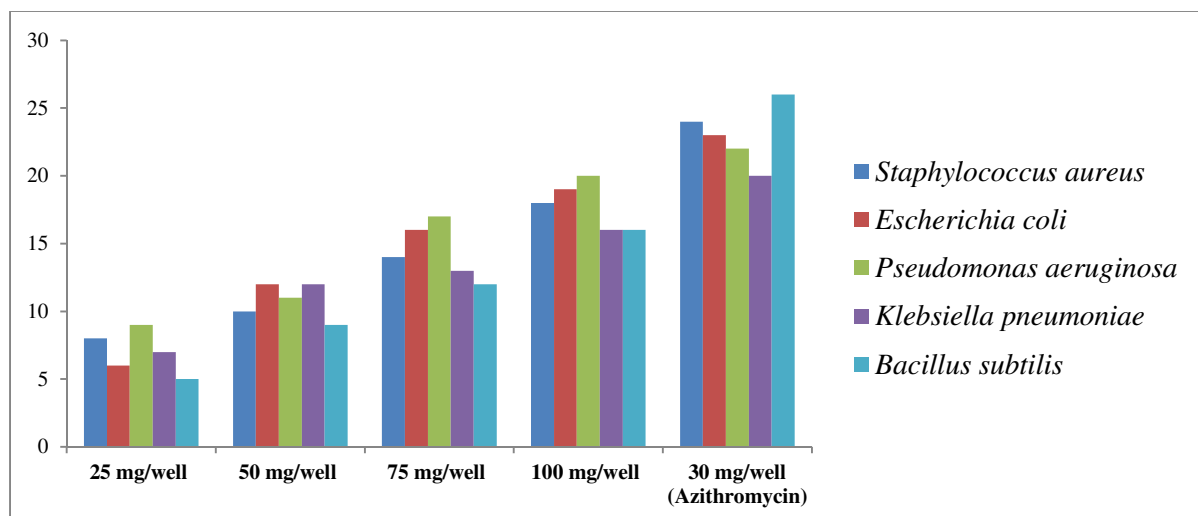
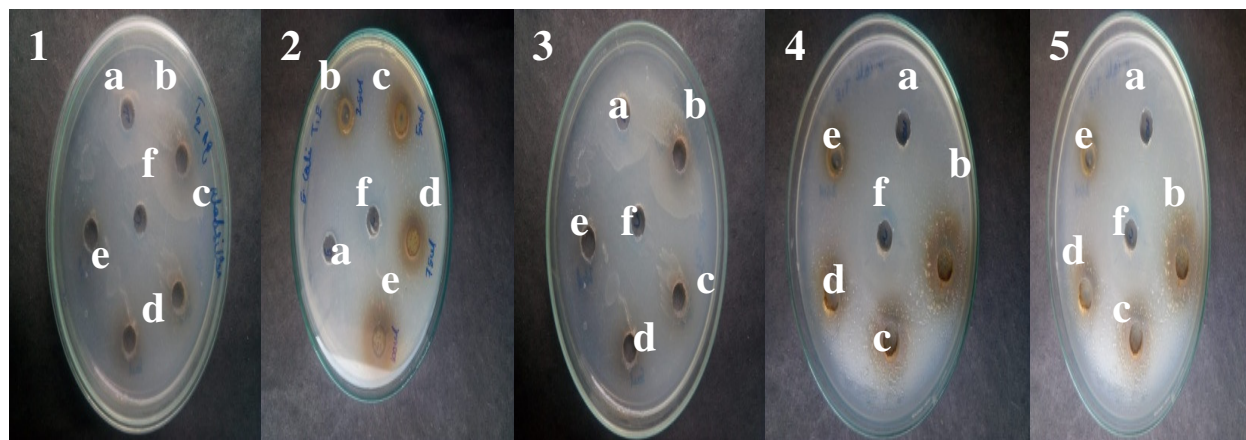
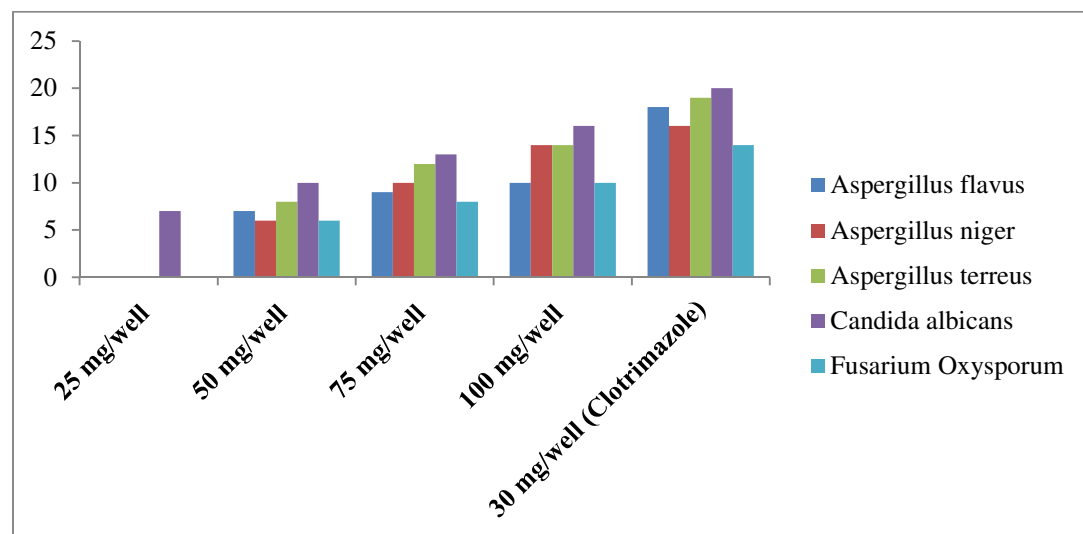
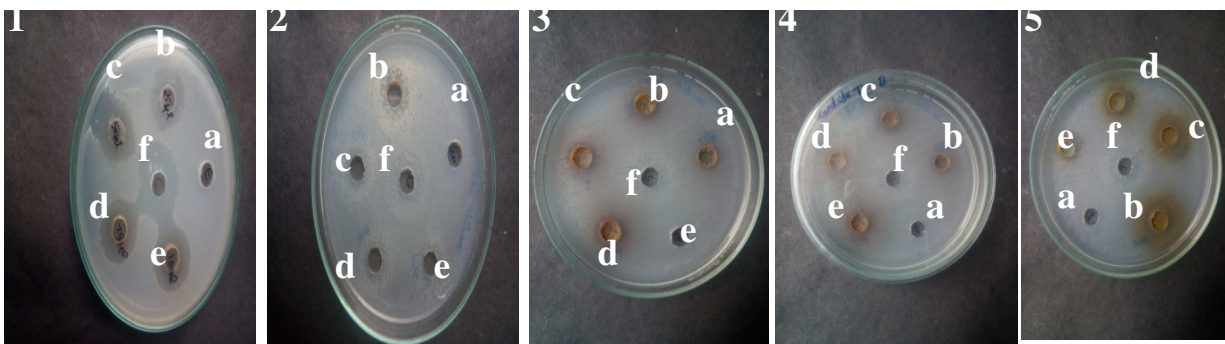
Fig.-7: Antibacterial Activity of *Xanthium strumarium* NPsFig.-8: *In vitro* antibacterial activity: 1. *Staphylococcus aureus*, 2. *Escherichia coli*, 3. *Pseudomonas aeruginosa*, 4. *Klebsiella pneumoniae*, 5. *Bacillus subtilis*[a: 0 μ g/well; b: 25 μ g/well; c: 50 μ g/well; d: 75 μ g/well; e: 100 μ g/well; f: 30 μ g/well (Azithromycin)]

Fig.-9: Antifungal Activity of Nanoparticle Sample

Table-2: Antifungal Activity of Nanoparticle Sample

S. No.	Name of Organisms	Zone of Inhibition(mm)				
		25	50	75	100	Clotrimazole (30)
1	<i>Aspergillus flavus</i>	-	7	9	10	18
2	<i>Aspergillus niger</i>	--	6	10	14	16
3	<i>Aspergillus terreus</i>	-	8	12	14	19
4	<i>Candida albicans</i>	7	10	13	16	20
5	<i>Fusarium Oxysporum</i>	-	6	8	10	14



1. *Aspergillus flavus*, 2. *Aspergillus niger*, 3. *Aspergillus terreus*, 4. *Candida albicans*, 5. *Fusarium Oxysporum*
[a: 0 µg/well; b: 25 µg/well; c: 50 µg/well; d: 75 µg/well; e: 100 µg/well; f: 30 µg/well (Clotrimazole)]

Fig.-10: *In vitro* Antifungal Activity

CONCLUSION

In summary, a green, novel synthesis of gold nanoparticle bio-fabricated with *Xanthium strumarium* leaves extract is achieved in the first time. The protocol presented in this work is very simple, greener and no need capping agent. The synthesized nanoparticles are well characterized using suitable methods. The shape of the nanoparticle is spherical shape and range from 9.60 nm to 11.70 nm. *In vitro* antimicrobial activity and *in vitro* antifungal also studied and found to have considerable activity.

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