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COMPARATIVE *IN-VITRO* STUDY OF ANTIMICROBIAL, ANTIFUNGAL OF SILVER NANOPARTICLES SYNTHESIZED FROM AQUEOUS AND ETHANOLIC LEAF EXTRACT OF *GMELINA PHILIPPENSIS* CHAM.

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Keywords:

Silver nanoparticles, Antimicrobial activity, Antifungal Activity

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ABSTRACT: The development of a dependable green chemistry approach for the biogenic production of nanomaterials is an essential component of contemporary nanotechnology research. The biosynthesis of metal nanoparticles is a widely acknowledged method that employs a bottom-up approach with reduction as the primary process. When compared to chemical approaches, the nanoparticles created by this method are safer, more cost-effective and less harmful to the environment. Metallic nanoparticles with bactericidal and inhibitory properties include silver nanoparticles. Pathogenic bacteria have become more resistant to antimicrobial medicines in recent years, which presents a significant challenge to the healthcare system. Additionally, the fast development of antibiotic resistance during this time has made scientists and researchers reconsider the therapeutic potential of silver and its nanoparticulate systems as possible antibacterial and antifungal agents. In the present investigation, we have comparatively studied the antimicrobial and antifungal activity of 1mM, 0.1 M solution of silver nitrate, silver nanoparticles synthesized from aqueous and ethanolic leaf extract and Cd- doped silver nanoparticles and antioxidant activity of aqueous and ethanolic extract of *Gmelina philippensis* Cham. For the antimicrobial activity we have used two gram-negative and two gram-positive pathogens. For antifungal activity, the fungus *Candida albicans* was used and DPPH method was used to examine the antioxidant activity.

INTRODUCTION: The most promising are the metallic nanoparticles, which exhibit strong antibacterial qualities due to their large surface-to-volume ratio [1]. This is concerning because research interest is fluctuating due to the emergence of safe strains, antitoxins, and microbial resistance to metallic particles [2].

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The physical characteristics of metallic particles in the nanometer range differ from those of the bulk material [3]. Because of their highly dynamic morphologies, they exhibit remarkable features such as expanded reactant mobility [4]. By reducing the amount of metal particles, microorganisms such as bacteria and organisms now play a crucial role in the cleanup of hazardous metals [5].

In pathogenic species, the AgNPs were successfully causing cell membrane polymer component disruption. Following this, the bacterial system's cell membrane is broken by nanoparticles' reciprocal action, which also disrupts the protein production mechanism [6].

Silver nanoparticle concentrations that are higher than lesser concentrations have a faster membrane permeability, which causes bacteria to burst their cell walls [7]. The highest conductivity was found in *Rhizophora apiculata* reduced silver nanoparticles, which also had fewer bacterial colonies than $AgNO_3$-treated cells in the experimental plate [8]. This is likely because the smaller particle size and greater surface area of the reduced silver nanoparticles cause increased membrane permeability and cell death [9].

According to Scandalis, the interactions between bacteria and metallic silver and gold nanoparticles bind with the active site of cell membranes and inhibit cell cycle processes [10]. Enicostemma littorale extract was used as a reducing and capping agent to produce the biosynthesized silver and zinc nanoparticles in a single step [11]. The plant extract successfully reduced silver nanoparticles and demonstrated efficacy against *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* (gram-negative), and *Staphylococcus aureus* (gram-positive) and *Candida albicans* [12].

The silver nanoparticles synthesized using aqueous and ethanolic extract of *Gmelina philippensis* Cham. was used to investigate the antimicrobial, antifungal activity. The 1mM, 0.1 M conc. of silver nitrate solution and silver nanoparticles synthesized from ALEGP and ELEGP as well as Cd doped silver nanoparticles were studied for antimicrobial, antifungal and antioxidant activities. All the pathogens tested during analysis are human pathogens.

MATERIALS AND METHODS:

Collection of Plant Materials: The healthy and fresh plant leaves were collected from the Botanical Garden of the Govt. Vidarbha Institute of Science and Humanities Amravati (M.S., India) during the rainy season. The collected plant leaves were cleaned and thoroughly washed with double distilled water before being dried for 15 days at room temperature. The dried leaves were crushed to a coarse powder and kept in an airtight container for future use.

Chemicals and Reagents: Deionized water, ethanol, silver nitrate, cadmium nitrate, DPPH.

Preparation of the *Gmelina philippensis* Cham. Extract: A mixer grinder is used to powder the air-dried plant leaves. Using a Soxhlet extractor, 10 g of powdered leaves were extracted in water and ethanol. For extraction, 250 ml of each solvent was mixed with 10 gm of powdered leaves and heated for 7 hours at about 50-60^{0}C as shown in **Fig. 1**. Following complete solvent evaporation, each of these solvent extracts was dried, weighed and stored at room temperature in an airtight bottle until further analysis.

FIG. 1: PREPARATION OF PLANT EXTRACT IN SOXHLET APPARATUS (A) AQUEOUS EXTRACT (B) ETHANOLIC EXTRACT

HPLC (High Performance Layer Chromatography): The apparatus consists of a solvent reservoir, sample injector, pressure pump, HPLC tube and diode detector. The process begins

by injecting the extracts to be separated at the bottom of the HPLC. In addition, a suitable solvent is poured into the solvent reservoir. The tap is now opened to allow the movement of solvent downward, which is then pushed by a pressure pump to mix up with the injected sample. Finally, the extracts moved into the diode detector, which separated the compounds, removed the waste and pumped the final content to processing units.

Synthesis of Silver Nanoparticles Using Aqueous, Ethanolic Extract of *Gmelina philippensis* Cham. and Cadmium Doped Silver Nanoparticles: The silver nanoparticles were synthesized by mixing of 1mM solution of silver nitrate to each 5 ml of aqueous extract and ethanolic extract. After addition there was a change in color that indicates the formation of silver nanoparticles. The cadmium doped silver nanoparticles was also synthesized by adding of 1mM solution of silver nitrate to 5 ml of aqueous extract and 5 ml of cadmium nitrate solution as shown in **Fig. 2-4**. The synthesized nanoparticles were further confirmed by UV-Vis. spectroscopy, XRD, TEM etc.

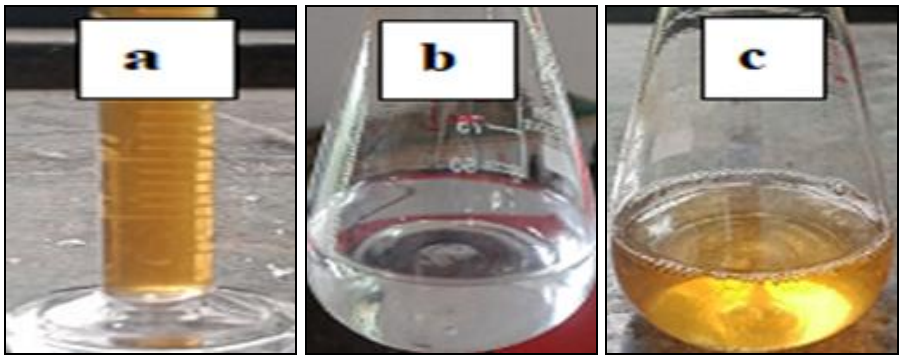

FIG. 2: VISUAL CHANGE OF COLOR AFTER ADDITION OF LEAF EXTRACT TO SILVER NITRATE, (A) AQUEOUS LEAF EXTRACT, (B) SILVER NITRATE (C) JUST AFTER ADDITION

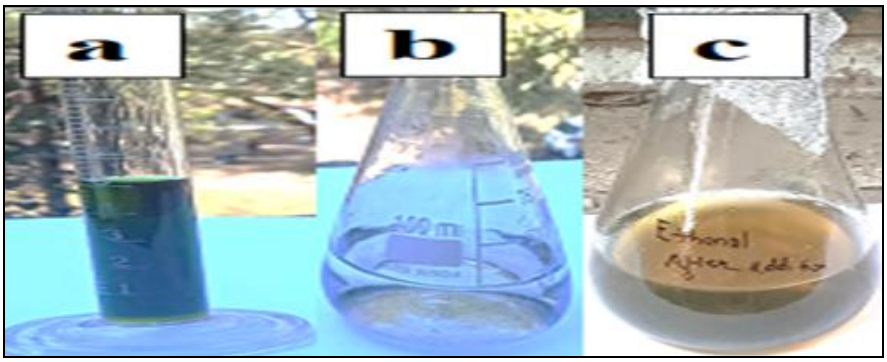

FIG. 3: VISUAL CHANGE OF COLOR AFTER ADDITION OF LEAF EXTRACT TO SILVER NITRATE, (A) ETHANOLIC LEAF EXTRACT, (B) SILVER NITRATE (C) JUST AFTER ADDITION

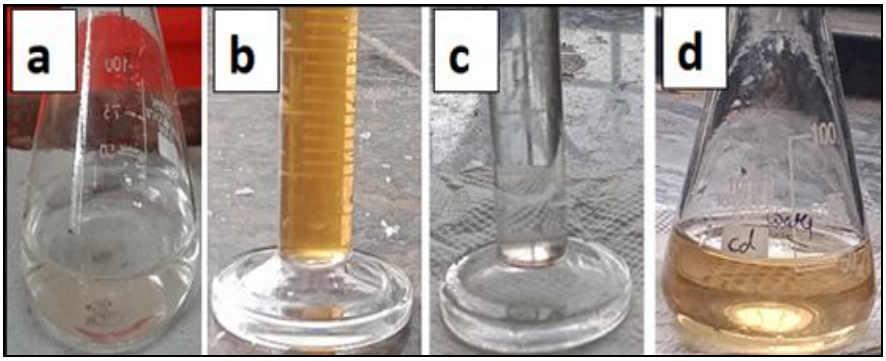

FIG. 4: VISUAL OBSERVATION OF CD-DOPED SILVER NANOPARTICLES AFTER ADDITION OF LEAF EXTRACT AND CADMIUM NITRATE SOLUTION TO $AGNO_3$ SOLUTION AT DIFFERENT TIME INTERVALS (A) 1MM$AGNO_3$ SOLUTION (B) LEAF EXTRACT (C) 0.01PPM SOLUTION OF CADMIUM NITRATE (D) JUST AFTER ADDITION

Antibacterial Activity: The disc diffusion method was used to examine the antibacterial activity of the AgNPs synthesized from the aqueous and ethanolic leaf extract of *Gmelina philippensis* Cham. as well as 1mM, 0.1 M solution of silver nitrate solution and Cd-doped silver nanoparticles against *E. coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus or Enterococcus faecalis*. On nutrient agar media at a temperature of 37 °C, the pure culture of microorganism was subcultured. On each plate, a sterile cotton swab was used to evenly swab each strain. For the bacterial colonies to proliferate at their fastest rate, the culture was incubated for 72 hours. Using gel puncture, 6 mm-diameter wells were created on the nutritional agar substrate.

Antifungal Activity: Using the Kirby-Bauer agar-well disc diffusion method, the antifungal effects of Ag nanoparticles from the aqueous and ethanolic leaf extract of *Gmelina philippensis* Cham. and Cd-doped silver nanoparticles were studied. *Candida albicans* was assembled as a stock fungal strain and kept maintained in media solution. Dextrose, peptone, $NH_4H_2PO_4$, KNO_3, $CaCl_2$, and agar were dissolved in double-distilled water to create the *Candida albicans* media. The prepared media was then autoclaved for 15 minutes at 121 degrees Celsius under 15 pounds of pressure. In the antifungal experiment, the common drug itraconazole served as a positive control for drug-induced death. A microtiter plate was used to measure absorbance (OD) at 600 nm in order to calculate the amount of fungal growth.

RESULT AND DISCUSSION:

HPLC: For HPLC, the ethanolic and aqueous extract was used. These extracts are denoted as 1, 2 as shown in **Fig. 5.** Toluene and ethyl acetate were used in a ratio of 7:3 to develop the HPLC plate. A 10% aluminium chloride solution was sprayed on the compounds to help them become visible. It was noted that pink or blue color spots were obtained from the HPLC chromatogram.

FIG. 5: HPLC OF EXTRACTS IN DIFFERENT SOLVENTS

TABLE 1: RF VALUE OF EACH EXTRACT IN DIFFERENT SOLVENT

Sr. no.	Sample code	Extract	No. of spots	R_f values
1	1	Ethanol extract	3	(1) 0.051 (2) 0.26 (3) 0.92
2	2	Aqueous extract	1	(1) 0.076

The R_f value was calculated by using the formula distance travelled by the solute to the distance travelled by the solvent. The distance travelled by the solvent is 7.8 cm. The ethanolic extract showed 3 spots at 0.051, 0.26 and 0.92 as depicted in table 1. The aqueous extract revealed only one spot at 0.076. The Ethanolic extract showed spot of blue color at R_f value 0.92 which was considered as flavonoids.

Antimicrobial Activity:

Activity against *E. coli:* *E. coli* is a gram negative pathogen living only in human or animal intestine. The clinical infection caused by *E. coli* were urinary tract infection, diarrhoea, pathogenic infection and septicemia [13]. The presence or absence of inhibitory zones was used to determine the antibacterial potency. The findings revealed that the silver nanoparticles synthesized from

ALEGP, ELEGP and 1 mM solution of silver nitrate does not showed inhibitory activity against *E. coli* whereas 0.1 M solution of silver nitrate and Cd-doped silver nanoparticles are active against *E. coli*. The zone of inbition for 0.1 M solution of silver nitrate and Cd-doped silver nanoparticles are found to be 12mm and 20mm.

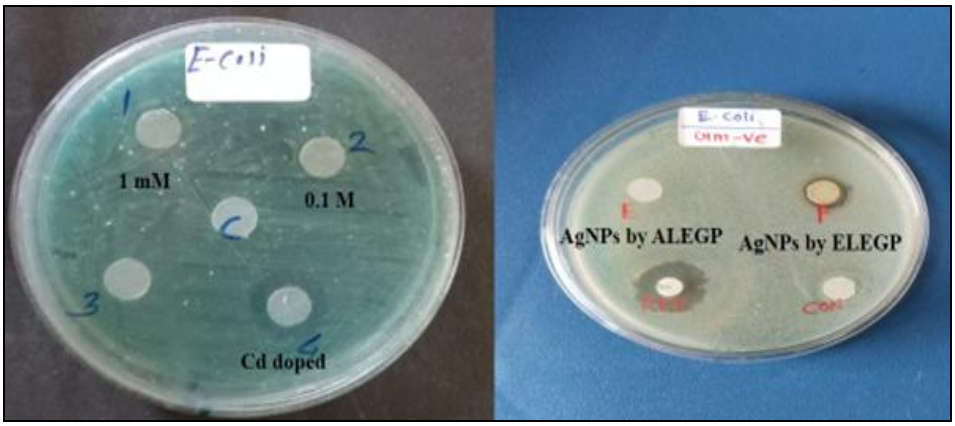

FIG. 6: ANTIBACTERIAL ACTIVITY OF 1MM CONC. OF $AGNO_3$, 0.1 M CONC. OF $AGNO_3$, CD- DOPED AGNPS AND SILVER NANOPARTICLES SYNTHESIZED USING ALEGP, ELEGP AGAINST *E. COLI*

Activity against *K. pneumoniae*: *K. pneumoniae* is a gram negative bacteria that can cause different types of healthcare associated infections, including pneumoniae, bloodstream infections, wound or surgical site infections and meningitis [14]. No antibacterial effect against K. pneumoniae was observed for silver nanoparticles synthesized from aqueous extract, 1mM and 0.1 M solution of silver nitrate solution whereas nanoparticles synthesized using ELEGP and Cd- doped silver nanoparticles are active against *K. pneumoniae.* The zone of inhibition for nanoparticles synthesized using ELEGP, and Cd- doped silver nanoparticles are found to be 24mm, 19mm.

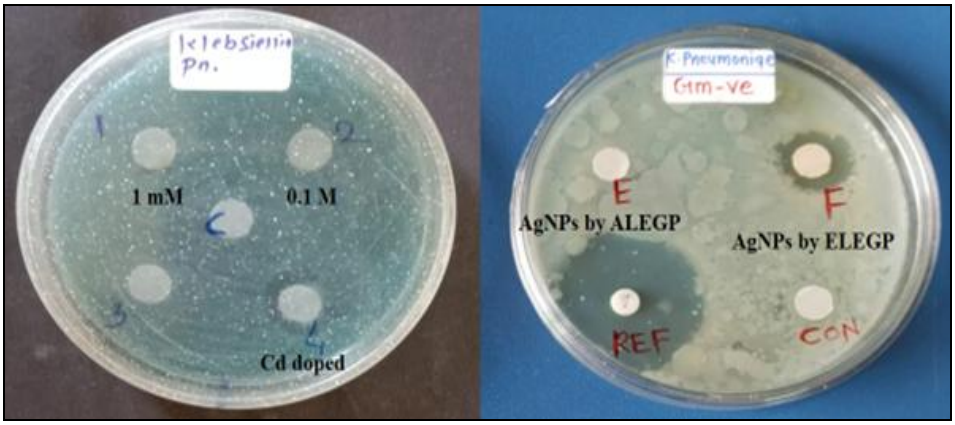

FIG. 7: ANTIBACTERIAL ACTIVITY OF 1MM CONC. OF $AGNO_3$, 0.1 M CONC. OF $AGNO_3$, CD- DOPED AGNPS AND SILVER NANOPARTICLES SYNTHESIZED USING ALEGP, ELEGP AGAINST *K. PNEUMONIAE*

Activity against *S. aureus*: *S. aureus* is a gram-positive bacteria that causes wound infection. It grows in bunches, similar to grapes. Its propensity to acquire resistance to penicillin and other antibiotics contributes to its significance as a human disease [15].

It causes infection and intoxication. The former is caused by an infected host. Intoxication produced by Staphylococcal infections in the host. Staphylococcal infections are among the most prevalent bacterial illnesses, and they can range from minor to fatal [16]. Because such bacteria develop resistance to ordinary antibiotics, newer and newer medications must be created and tested against them all the time.

From **Table 2** it was observed that silver nanoparticles synthesized from aqueous extract, 1mM and 0.1 M solution of silver nitrate solution does not show antimicrobial activity against *S. aureus* whereas nanoparticles synthesized using

ELEGP and Cd- doped silver nanoparticles are active against *K. pneumoniae*. The zone of inbition for nanoparticles synthesized using ELEGP, and Cd- doped silver nanoparticles are found to be 12mm and 13mm.

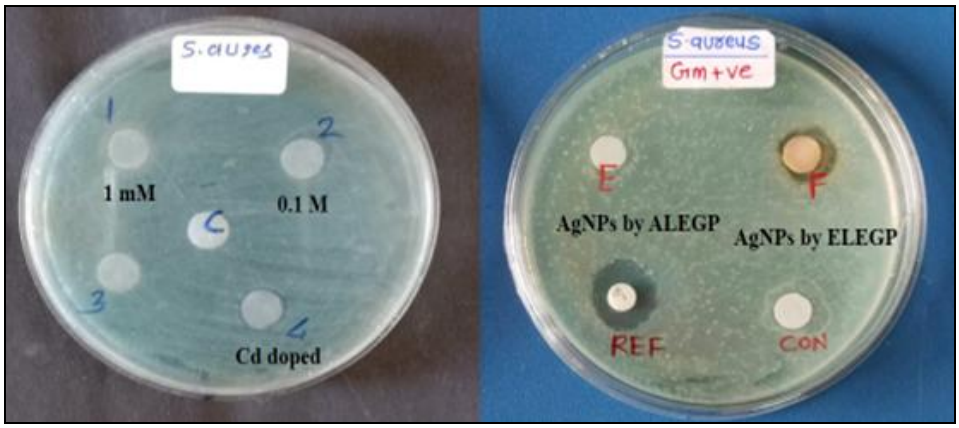

FIG. 8: ANTIBACTERIAL ACTIVITY OF 1MM CONC. OF $AGNO_3$, 0.1 M CONC. OF $AGNO_3$, CD- DOPED AGNPS AND SILVER NANOPARTICLES SYNTHESIZED USING ALEGP, ELEGP AGNPS AGAINST *S. AUREUS*

Activity against *S. faecalis:* *S. faecalis* is a gram positive, commensal bacterium inhabiting the gastrointestinal tracts of humans [17]. It is found in healthy humans and can be used as a probiotic [18, 19]. From **Table 2,** it was observed that *S. faecalis* are active against silver nanoparticles synthesized from ALEGP, ELEGP, 1mM and 0.1 M solution of silver nitrate solution and Cd-doped silver nanoparticles. The zone of inhibition was found to be 13mm, 11mm, 13mm, 13mm and 18mm.

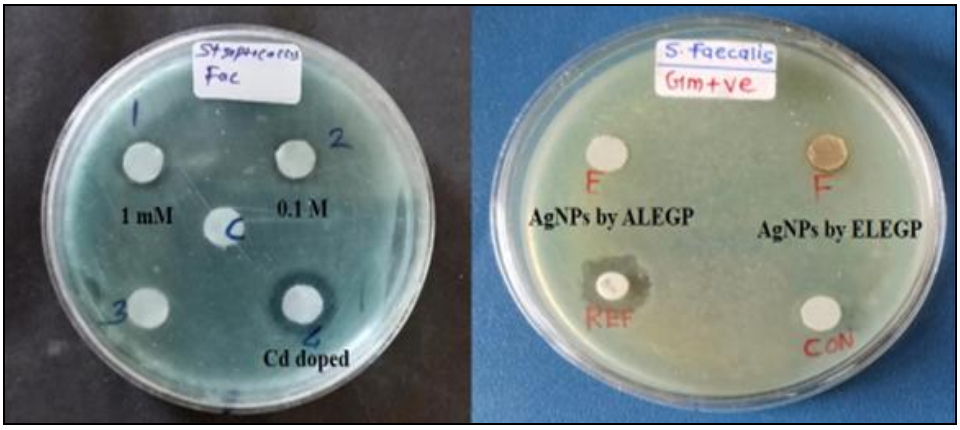

FIG. 9: ANTIBACTERIAL ACTIVITY OF 1MM CONC. OF $AGNO_3$, 0.1 M CONC. OF $AGNO_3$, CD- DOPED AGNPS AND SILVER NANOPARTICLES SYNTHESIZED USING ALEGP, ELEGP AGAINST *S. FAECALIS*

TABLE 2: ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED FROM ALEGP AND ELEGP, 1MM, 0.1M CONC. OF SILVER NITRATE SOLUTION AND CD- DOPED SILVER NANOPARTICLES

Sr. no.	Sample	*E. coli*	*K. pneumoniae*	*S. aureus*	*S. faecalis*
1.	Silver nanoparticles synthesized using ALEGP	---	---	---	13mm
2.	Nanoparticles synthesized using ELEGP	---	24mm	12mm	11mm
3.	1mM conc. of $AgNO_3$	---	---	---	13mm
4.	0.1 M conc. of $AgNO_3$	12mm	---	---	13mm
5.	Cd-doped AgNPs	20mm	19mm	13mm	18mm

Antifungal Activity: Fungi cause a variety of serious diseases and treatment of such infections is critical because commonly available medications (amphotericin B, Nystatin, itraconazole, and so on) used for therapy produce severe side effects such as liver and renal damage [20]. Kirby - Bauer agar-well disc diffusion method **Fig. 10** was used to obtain antifungal pursuit of both nanoparticles.

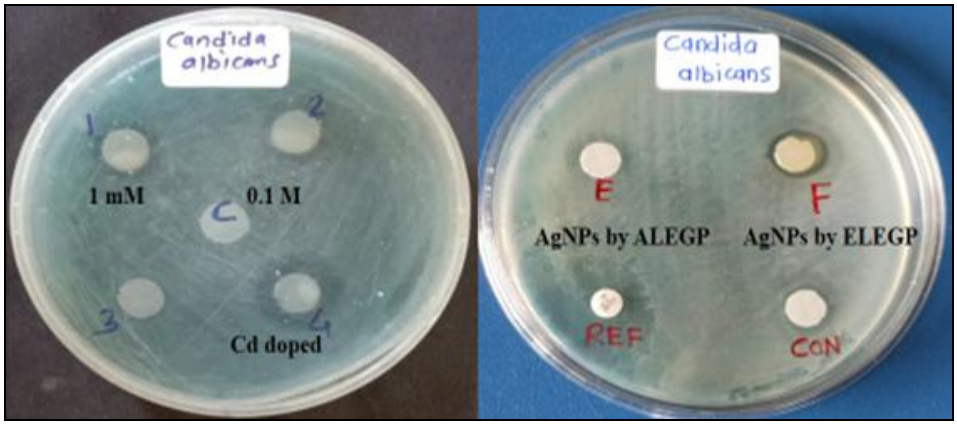

FIG. 10: ANTIFUNGAL ACTIVITY OF 1MM CONC. OF $AGNO_3$, 0.1 M CONC. OF $AGNO_3$, CD- DOPED AGNPS AND SILVER NANOPARTICLES SYNTHESIZED USING ALEGP, ELEGP AGAINST *CANDIDA ALBICANS*

Candida albicans was the fungal pathogen against which the efficiency of biosynthesized silver nanoparticles, 1mM. 0.1M solution of silver nitrate and cadmium doped silver nanoparticles was investigated. The inhibitory zoneof 1mM, 0.1 M, cadmium doped silver nanoparticles and nanoparticles synthesized using ALEGP and ELEGP is 11mm, 11mm, 1mm, 10mm and 22mm.

TABLE 3: ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES USING ALEGP, ELEGP, 1MM CONC. OF $AGNO_3$, 0.1 M CONC. OF $AGNO_3$ AND CD- DOPED AGNPS AGAINST *CANDIDA ALBICANS*

Sr. no.	Sample	*Candida albicans*	Zone of inhibition
1.	Silver nanoparticles synthesized using ALEGP	Active	11mm
2.	Nanoparticles synthesized using ELEGP	Active	11mm
3.	1mM conc. of $AgNO_3$	Active	11mm
4.	0.1 M conc. of $AgNO_3$	Active	10mm
5.	Cd- doped AgNPs	Active	22mm

CONCLUSION: The presence of flavonoids in aqueous, ethanolic was confirmed by HPTLC. This plant appears to be a promising drug for new drug discoveries. In this study we compared the antimicrobial and antifungal activity of silver nitrate solution with synthesized silver nanoparticles and we found that synthesized silver nanoparticles enhanced the antimicrobial and antifungal activity.

The study discovered that Cd-doped silver nanoparticles synthesized from ALEGP have strong antibacterial and antifungal activities. These silver nanoparticles have the potential to be extremely useful in the pharmaceutical and biomedical industries.

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