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Pharmacological Activity Of Green Synthesized Silver Nanoparticles Of Argemone Mexicana As Antifungal Agent

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Abstract:
Antimicrobial resistance development has grown to be a serious health issue. Therefore, the need to find better, more powerful tactics to combat Antimicrobial resistance remains ongoing. Silver nanoparticles (AgNPs) possess unique physico-chemical and excellent antifungal properties. AgNPs have been shown to have antifungal properties in numerous investigations. So, the budding potential of AgNPs as antifungal should be a positive indication to combat Antimicrobial resistance . The green method of AgNP production using plant material has several advantages: freedom from toxic chemicals, cost effectiveness, simplicity, easy scalability, economic viability, and eco-friendliness. AgNPs have been synthesized using different parts of plants, including fruit, seed, bark, peel, root, and leaf. In order to successfully reduce the silver salt and stabilize/cap the nanoparticles, secondary metabolites present in plant extract, such as polyphenols, terpenoids, flavonoids, proteins, saponins, and vitamins, are required. In addition to their role in the efficient synthesis of AgNPs, synergistic interaction between the plant extract and AgNPs might result in a marked increase in the observed antifungal activity. Based on their phytochemical profile and antimicrobial activity A. maxicana selected to synthesize AgNPs. Moreover, forming AgNPs using the plants may synergize between nanoparticles and plant extracts for antifungal activity.
Keywords: *Nanoparticles, AgNPs , Antimycotics, Argemone mexicana ,Flavonoids Antimicrobials*

1. INTRODUCTION:

Plant based antimicrobials represent a vast untapped source of medicine and they have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. *Candida* bloodstream infections steadily .Since no fungal vaccines are currently licensed, the only clinical recourse to combat invasive mycoses is the use of antifungal therapeutics (antimycotics). Treatment of invasive mycoses by some of these antimycotics is hampered because of the limited activity spectrum, resistance development, and fungistatic rather than fungicidal activities. Some isolates of *C. glabrata*, for instance, display low fluconazole susceptibility. *C. albicans* infections faces a number of problems including limited number of effective antifungal agents, toxicity of the available antifungal agents, resistance of *Candida* to commonly used antifungals, relapse of *Candida* infections and non-cost effective antifungal agents . *C. albicans* is a major fungal pathogen responsible for the majority of nosocomial infections . It causes infection in its biofilm mode of growth and has taken centre stage with the increasing recognition of its role in human infections due to the development of resistant or phenotypic adaptation within the biofilm . Since years, azole drugs and their derivatives continue to dominate as antifungal agents of choice against *Candida* related infections, as topical applications, or as oral drugs.In addition, the use of oral antibiotics, broad-spectrum antibiotics , corticosteroids, or immunosuppressives may predispose individuals to infection. These factors disrupt the balance between the healthy host and the *Candida*, which under normal circumstances, may be present in the gastrointestinal flora but is not disease producing . Emergence of drug resistant strains and dose limiting toxic effects has further complicated the treatment of infectious diseases. These complications have necessitated the search for new antimicrobial substances from various sources. The search for new drugs, with antifungal activity, has been a constant preoccupation of the researchers, toward pathological studies caused by these microorganisms.[1,2]

2. Nanotechnology

Nanoparticles (NPs) are particles having one of their dimensions ranging in size from 1-100 nm. As the nanoparticles get smaller, their surface area increases, which significantly changes their chemical, biological,

and physical properties. Organic and inorganic are the two types of NPs that can be created. Metallic NPs (such as Cu, Ag, Au, and Al), semi-conductor NPs (such as ZnS, ZnO, and CdS), and magnetic NPs (such as Fe, Co, and Ni) are examples of inorganic nanoparticles. In contrast, carbon nanoparticles (such as quantum dots and carbon nanotubes) are examples of organic nanoparticles. Silver is the most favoured metal in biological systems among all. Due to the unique electrical, magnetic, optical, mechanical, chemical, and size-dependent properties of these noble metal nanoparticles, recent research has focused on these particles. They show significant differences from the bulk materials from which they form. The metal nanoparticles find wide applications in optoelectronics, biomedical, electronics, information storage, and magnetic systems owing to their size-dependent properties. Due to their exceptional and surprising catalytic, chemical, mechanical, electronic, and thermal properties, differing substantially from the bulk materials, nanoscale materials have grabbed significant attention .[3,4]

Silver has been used for several applications, particularly for antimicrobial protection. Silver-based antiseptic formulations have been conventionally used for controlling the growth of bacteria in burn healing, disinfection of medical equipment, and dentistry . Due to their distinctive physico-chemical properties and outstanding antimicrobial properties, AgNPs are the most favoured NPs. They find several applications in hygiene, building materials, drug delivery, biomolecular detection, biosensing, water treatment, catalysis, textile industry, imaging, and many other biomedical and engineering applications, food processing, cleaning, and disinfection, textiles, and medical products. They also have wound healing, anti-acne, and anti-dandruff properties. The silver nanoparticles have shown tremendous scope in different fields like biosensors, catalysis, biomolecular detection and diagnostics, agriculture, waste treatment, drug delivery, and food production. In addition, silver nanoparticles have demonstrated other biological applications like anti-inflammatory, antimicrobial, anticancer, antioxidant, antidiabetic, and antiparasitic.[5,6]

Various physico-chemical processes are employed to synthesize AgNPs . But they suffer from several drawbacks like high temperature, energy, pressure, and hazardous chemicals posing a significant risk to both humans as well environment. In recent times, the studies have become much focused on the green method of AgNP production using plant material which comes with several advantages like freedom from toxic chemicals, cost-effectiveness, simplicity, easy scalability, economic viability, and eco-friendliness. Different plant parts, including fruit, seeds, bark, peel, roots, and leaves, have been used to create AgNPs .The effective reduction of Ag salt and capping /stabilization of the formed AgNPs depends on secondary metabolites in plant extract, such as polyphenols, terpenoids, flavonoids, saponins, vitamins, and proteins . Plants selected for the present study have shown antimicrobial activity and many other significant medicinal activities. Phytochemical-assisted reduction is the fundamental mechanism underpinning plant-mediated AgNP production. The main phytochemicals include terpenoids, ketones, flavones, amides, aldehydes, and carboxylic acids. Examples of aqueous-soluble phytochemicals that aid in the fast reduction of Ag^+ ions include organic acids, quinones, and flavones. Xerophytes include emodin and anthraquinone, which go through tautomerization and produce AgNPs . The plant extract serves a dual role as a reducing and a stabilizing/capping agent agent negating the requirement of an external stabilizing/capping agent. The suggested mechanism for producing AgNPs from plant phytochemicals involves the transfer of charge from the reductant to $Ag^{+,}$ causing the formation of Ag atoms followed by nucleation to produce tiny AgNPs. After that, the small particles enlarge to produce larger ones, and finally, adsorption of surplus negatively charged reducing agents. Large capping molecules cause steric hindrances, which cause the AgNPs' size to be more stable.[7,8]

3.Biological methods of AgNPs synthesis:

Traditional NP production methods are expensive, toxic, and environmentally harmful. Researchers have pinpointed the exact green pathways, or naturally occurring sources and materials, that can be used to produce AgNPs in order to address these problems. Green synthesis of AgNPs can be classified into three types: (a) microbial species like bacteria, fungi, yeasts, and actinomycetes, (b) whole plants or their extracts, and (c) virus DNA, membranes, and diatoms . Bacterial approaches are not commercially feasible since they require highly sterile conditions and upkeep. Additionally, synthesis is slow compared to techniques using plant-based materials, and only a small selection of sizes and shapes are bendable. Due to the low cost and convenience of

handling biomass, fungi-based nanoparticle production is widely used. Still, the genetic mutation of the organism may result in increased contamination. Furthermore, the synthesis rate is insufficient to deal with total biomass production . A lot of focus has been placed on the environmentally friendly synthesis of AgNPs utilizing plant material because of its many benefits, including simplicity in preparation, lack of harmful chemicals, affordability, and eco- friendliness. Flavonoids, phenolic acids, proteins, terpenoids, and amino acids are among the phytochemicals responsible for reducing Ag^{+} to Ag^{0} and capping the resulting AgNPs.[9-11]

4.Argemone mexicana L.

Mexicana is a native plant of Mexico and now widely spread to tropical and subtropical regions of the world. The plant commonly found as a weed in agricultural fields, waste lands and road side in India. *A. mexicana* belongs to Papaveraceae family. Mexicana is an annual herb, in India the plant starts budding in early winter and disappears during the late summer. The weed can survive in nutritionally poor soil and grows up to 0.5 to 1 meters of height, leaves are in greenish color with gray white prickles, and leaves area round 6 to 12 cm long and flowers are located at tip of branches, yellow in color of 4 to 5 cm in diameter. It's a prickly plant all the parts of plant has prickles except root. The seeds are spherical, covered with veins and about 2to 3 cm in length. The *Argemone mexicana linn.* is useful in guinea- worm infestations, purgative and diuretic. Seeds of the plant are used as an antidote in snake poisoning and also acts as an emetic, expectorant, demulcent and laxative. The protein-dissolving substances containing seed extract is used to cure warts, cold sores, cutaneous infections, itches, jaundice and dropsy . Seeds are effective against skin infection, sores, dropsy and jaundice . Juice of the plant cures ophthalmic and opacity of cornea. Oil of the seed is used to treat skin diseases. Roots are antihelmentic and also used in, skin diseases, leprosy and inflammations. The various parts of the plant have been reported for medicinal uses, some of them are highly effective. The pharmacological investigations done by various researchers on A. mexicana were given below: Antidiabetic activity ,Anti malarial activity, Wound Healing activity ,Hepatoprotective activity, Antihelmintic activity , Antiplasmodial activity, Larvicidal activity, Anticancer activity, Vasorelaxant activity, Antiasthmatic activity, Cytotoxic activity, Anti-HIV activityand Molluscicidal activity.[12-15]

5.METHODOLOGY

Spectrophotometer Quantification of Total Phenolic Content:

Folin Ciocalteu reagent was intended for estimation of total phenolic content. Gallic acid was taken as standard for the determination of phenolic content and it was asserted as mg/g Gallic acid equivalent (GAE). Different combination ranging from about 0.01-.05 mg/ml of Gallic acid was processed in methanol. Concentration of plant extract was 1mg/ml using methanol as diluent. About 0.5ml of test sample was placed in a test-tube and admixed with 2.5ml of Folin Ciocalteu reagent. Sodium carbonate 2 ml of (7.5%) was added. The tubes were granted to standpoint for 30 minutes at room temperature, covered with paraffin, followed by taking the absorbance at 760 nm. Reading was taken in triplicate. Blue coloration was observed and measured spectrophotometrically.[16-18]

Spectrophotometric Quantification of Total Flavonoid Content:

Total flavonoids were measured by a colorimetric assay . An aliquot of sample (1mg/ml) or standard solutions (10-50µg/ml) of rutin was added to a 75 µl of sodium nitrite ($NaNO_2$, 5%) solution, and mixed properly. After 5-6 minute, 0.15 ml aluminium chloride ($AlCl_3$, 100 g/L) was added, followed by addition of sodium hydroxide 0.5 ml (NaOH, 4%) after 5 min. Volume adjustment was done upto 2.5 ml with distilled water and mixed thoroughly. Optical density was measured at 510 nm for sample, as well as blank. All samples were analyzed in three replications for sample and standard.[19,20]

Spectrophotometric Quantification of Total Alkaloid Content:

For the standard curve of atropine, total 5 different concentrations were used. 100 µg/ml solution was initially prepared from atropine (1 mg in 10 ml of distilled water). From this stock solution, exactly 0.5, 1, 1.5, 2 and 2.5 ml of atropine solutions was transferred to five different separating funnel. To each and individual funnels, 5 ml of phosphate buffer (pH4.7) and 5ml of Bromocresol green (BCG) solution was added and mixed

vigorously. The formed complex mixture is extracted with chloroform. Chloroform fraction was collected and final volume was adjusted in 10 ml volumetric flask with chloroform. Absorption at a wavelength of 470 nm of each flask was measured and calibration graph was drawn. Plant extract (1mg/ml) was admixed in HCl(2N) and filtered. The pH of the plant extract was fixed to neutral with 0.1 N NaOH. Solution(1ml) was transferred to a separating funnel and around bromocresol green(5ml) and phosphate buffer(5ml) was combined and mixed properly. The mixture was further extracted with chloroform (5ml) and transferred to 10 ml of volumetric flask and final volume was adjusted. The estimation was done by taking absorbance at 470 nm.[21,22]

Biological activity evaluation

Antifungal potentiality of leaf, stem and root extract:

Antifungal activity was screened of *Argemone mexicana linn.* leaf, stem and root extract by agar well diffusion method.Two fungi (*Aspergillus niger, Candidaalbicans)* was used for current research work. The fungi strain were grown on *Czepek dox agar.* Wells of 6mm diameters were expurgated from the medium and extracted 50 µl solution was added (100 mg/ml) into the well. Next day zone of inhibition was measured in mm. Fluconazole (10µg/ml) was taken as control.[23-25]

Biosynthesis of GAgNP of plant extract (stem):

10 ml of plant extract was added in 90ml of 0.01mol dm^{-3} $AgNO_3$ solution, the solution was kept for 24 hours at room temperature. The Synthesized silver nanoparticles were settled down in the beaker which was washed thrice with distilled water and centrifuged at 15000 rpm. The sample was finally dried at room temperature.[26]

Characterization Techniques of Nanoparticles:

Nanoparticles can be characterized by various approaches to ensure their size, shape; crystallite structure phase etc. characterization techniques used in this thesis are as follows[151]:

UV-Visible Spectroscopy:

Ultraviolet visible spectroscopy (UV-Vis Spectroscopy) is specifically absorption spectroscopy in the ultra violet visible region. It mainly consist of deuterium (200-350nm) and Halogen (300-1100) lamps for UV and visible/IR absorption respectivelyThe electrical excitation of deuterium at low pressure produces a continuous UV spectrum in the range 160-375nm.[27,28]

X-Ray Diffraction (XRD)

X-ray diffraction technique is a non-destructive characterization technique which provides detailed knowledge about the crystallite size and structure as well as chemical composition, and physical properties of the sample material. X-rays are electromagnetic radiation with wavelengths in the range 0.1-5A^{o} (equivalent to 125 KeV to 2.5 KeV) . XRD is mainly based on observing the scattered intensity of an X- ray beam striking a sample as a function of incident and scattered angle, polarization, and wavelength or energy. [29]

Determination of antifungal susceptibility:

Fungus in freeze dried condition and revived the culture on agar plate in laboratory. The antifungal activity was performed on petridishes containing potato dextrose agar when temperature of the medium reached at 40°C i.e. slightly above the room temperature. There was used lower concentration of 10, 30, 50 70, 90 µg/ml to determine zone of inhibition of synthesized silver nanoparticle on the fungal strains. The specific concentration (10, 30, 50 70, 90 µg/ml) of 100% stock solution silver nanoparticles (diluted in double distilled water) were added to petriplates of 9 cm diameter containing PDA. The rate of mycelial growth was measured after placing an active mycelial plug of fungi on petridishes. **Fluconazole** was used as positive control. Finally the petriplates were incubated at 28°C. The Diameter of Zone of inhibition was recorded after 48 hours.[30,31]

6. RESULTS

Qualitative analysis of phytochemical

Table 1: Qualitative analysis of different classes of phytochemical

Phytochemicals		Methanolic Extracts
Alkaloids	Mayer's	+
	Dragendroff's	+++

	Wagner's	++
Flavonoids	**Shinoda**	+
	Alkaline	+++
Phenolic Compounds	**Ferric chloride**	+++
	Lead acetate	++
Saponins		++
Sterols/ Steroids		++
Tannins		++
Glycosides		++

+ : less presence, ++ : moderate presence, +++ : high presence

Quantitative estimation of secondary metabolites

Three important secondary metabolites were extracted from the dried powdered material of *A. mexicana* and estimated. The maximum content estimated was total Phenols (5.98mg/ml) followed by Flavonoids (5.88mg/100mg) and Alkaloids (3.86mg/100mg)

Biological activity evaluation:

Antifungal potentiality of leaf, stem and root extract

Table 2: Antifungal potentiality of leaf, stem and root extract

Part	**Extracts**	**Aspergillus niger**	**Candida albicans**
		Zone of Inhibition(mm) against different extraction	
Leaf	**Methanol**	10.0	12.0
	Ethyl Acetate	9.0	11.0
	Aqueous	-	-
Stem	**Methanol**	15.0	18.0
	Ethyl Acetate	10.0	11.0
	Aqueous	-	-
Root	**Methanol**	11.0	16.0
	Ethyl Acetate	8.0	10.0
	Aqueous	-	-
Fluconazole(10µg/ml)		18.0	16.0

Plant mediated synthesis of AM-AgNPs and antifungal assay

Procedure of biosynthesis of AM-AgNPs:

10 ml of plant stem extract was added in 90ml of 0.01mol dm^{-3} $AgNO_3$ solution, the solution was kept for 24 hours at room temperature. The Synthesized silver nanoparticles were settled down in the beaker which was washed thrice with distilled water and centrifuged at 15000 rpm. The sample was finally dried at room temperature.

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Characterization of AM-AgNPs:

UV-Vis spectroscopy

UV-Vis spectroscopy was done by Unicam-5625 UV-Vis Spectrophotometer in University of Allahabad between the 300 nm to 1100 nm range. The absorption spectra of silver nanoparticles by green synthesis exhibited at 427cm.

Standard curve of the synthesized AM-AgNPs

The standard calibration curve of AM-AgNPs was used to calculate the concentration of drug released. Dilutions of 0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml were made and analyzed using UV-Vis spectrophotometer

X-Ray Diffraction

This technique provides effective finite crystallite size as broadening peaks, the crystallite size can be calculated . The Figure depicts the AM-AgNPs' XRD pattern. The face centre cubic (fcc) structure of silver can be inferred from the emergence of distinctive diffraction peaks at 38.14° (111), 44.41° (200), 64.54° (220), and 77.53° (311). The XRD pattern clearly revealed that the nanoparticles formed are crystalline in nature.

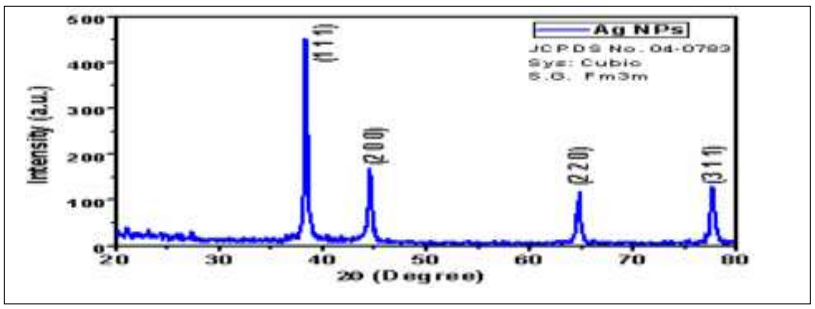

Fig 1: XRD graph of AM-silver nanoparticle

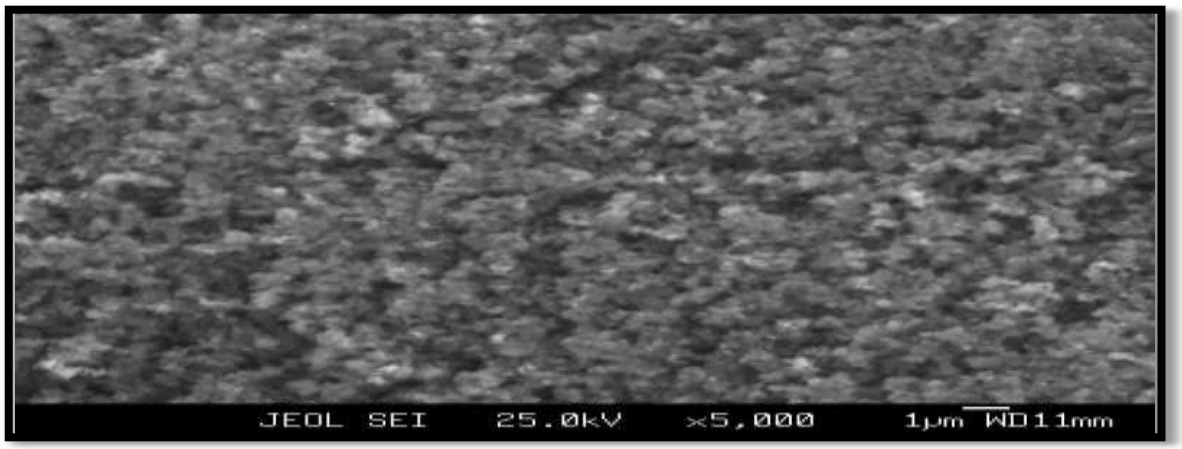

Fig 2: SEM image of AM-AgNP

Scanning electron microscopy: SEM analysis was performed by JEOL-JXA 8100 scanning machine. SEM micrograph shows spherical morphology of silver nanoparticle with agglomeration.

Transmission electron Microscopy

TEM analysis was done by JEOL JEM – 2100 Transmission Electron Microscope. Shape and size of the particle were more elucidated with the help of TEM. The TEM micrograph shows that the average size of the nanoparticles obtained was 58.2 nm.

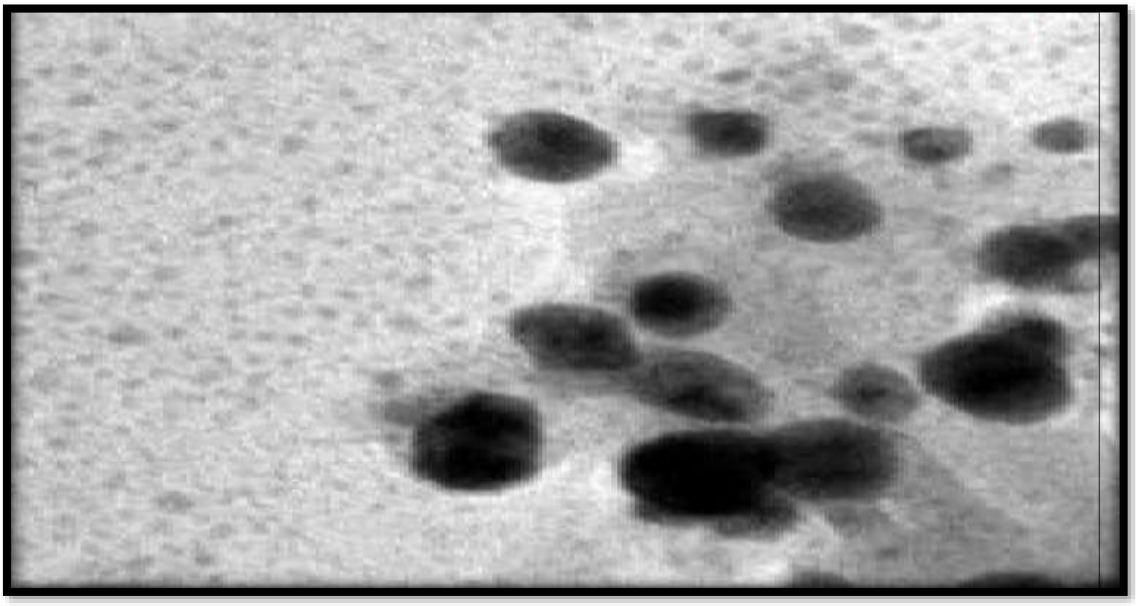

Fig3 : TEM image of chemically synthesized AM-AgNP

Determination of zeta potential: A crucial factor in determining the stability of aqueous nanosuspensions is the zeta potential. The stability of nanoparticles toward agglomeration will increase with increasing values of their zeta potential (either positive or negative) .The produced nanoparticles' zeta potential value was discovered to be -31.4 mV , showing that the particles had a respectable level of stability. PDI of the synthesized nanoparticles (0.147) was below 0.4, suggesting that the nanoparticles were almost monodisperse

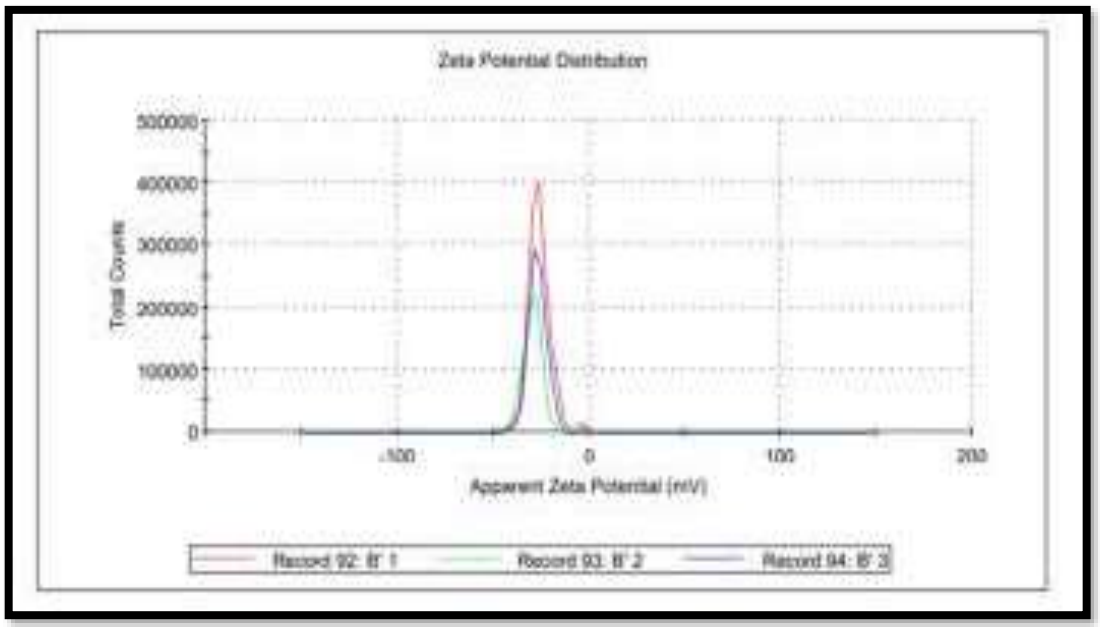

Fig 4: Zeta potential of the synthesized AM-AgNP

Determination of antifungal activity:

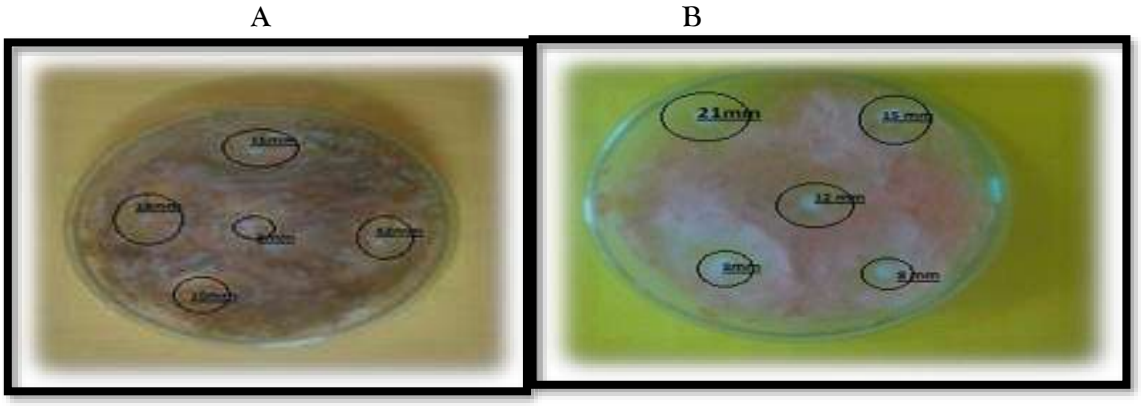

Fig 5: Antifungal evaluation of biologically synthesized silver nanoparticlesagainst (A): Aspergillus niger *(B):* Candida albicans

Table 3: Antifungal activity ofgreen silver nanoaprticles

	Fungal strains	**Aspergillus niger**	**Candida albicans**
Antifungal agent	**Concentration of AM-AgNp (µg/ml)**	**Zone of inhibition diameter (mm)**	
AM-AgNP	90	18	21
	70	15	15
	50	11	12
	30	10	08
	10	06	08
Control (Fluconazole)	**Negative**		**Positive**
	0		18

CONCLUSION:

Antimicrobial resistance (AMR) is now widely recognised as a serious health threat on a global scale. Several potential antimicrobial drugs performed significantly in the past have become redundant. Thus, the scientific community is constantly looking for novel, more efficient ways to tackle AMR. Due to their distinct physico-chemical characteristics and superior antibacterial activities, silver nanoparticles (AgNPs) have demonstrated promising outcomes in this regard among all other types of nanoparticles. The green method of AgNP production using plant materials has become more popular now days due to numerous advantages like freedom from toxic chemicals, cost-effectiveness, simplicity, easy scalability, economic viability, and eco-friendliness. Therefore, it is imperative that more are more AgNPs synthesized through green technology be screened for their antimicrobial potential. Antimicrobial evaluation was done against some pathogenic fungal by disc diffusion method Silver nanoparticles have potential antimicrobial activity towards many pathogenic microbes due to their large surface to volume ratio . Affinity of silver nanoparticles towards biological membrane provides the effective mechanism of antimicrobial activities of these structures . They get attached to the bacterial cell membrane and penetrate inside the bacteria. .

Today several pathogens are becoming resistant towards particular antibiotics which are causing fatal diseases in plants as wellas animals.. AM-AgNPs' may have an antifungal impact becausethey produce positively charged Ag+ ions, which interact with protein thiol groups to reduce membrane permeability and deactivate vital enzymes, leading to cell death.

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