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ORIGINAL ARTICLE

Green Synthesis and biological characterization of silver nanoparticles derived from *Erythrina variegate* and their antimicrobial assessment.

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ABSTRACT
Nanotechnology plays a significant role to influent the technological development with cost effective in the field of biochemical, environmental, pharmaceutical and other sectors of modern science. In our study, we synthesized silver nanoparticles (AgNPs) by reducing the fresh leaves of Erythrina variegate biologically. The synthesized silver nanoparticles are monitored under the UV Vis spectrophotometer which shows absorbance peak at 450 nm. Then, FTIR finded the functional biomolecules present which are responsible for reducing and capping.It shows the vibrational peaks at bands at 3395cm^{-1}, 3273cm^{-1}, 3190cm^{-1}, 3047cm^{-1}, and 2957cm^{-1}. The morphology and size of silver nanoparticles are analysed through SEM.TEM also observed to study the nature of nanoparticles. Later, the biosythesized silver nanoparticles shows antimicrobial activity against gram positive bacteria and gram negative bacteria and also antifungal activity.
Keywords: Nanotechnology, silver nanoparticles, SEM, TEM, UV, antimicrobial activity.

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INTRODUCTION

Therapeutic plants are one of the most significant aspects of our characteristic riches. Our progenitors were constrained to utilize any common substance that they could discover simply for their diseases, physical inconveniences, wounds, even serious sickness. One of the generally utilized indigenous information frameworks is the information and utilization of conventional medication. Such information, known as ethnomedicinal information. This study combines the traditional ethnomedicine with modern nanotechnology for the cost effective treatment in the modern world.

Nanotechnology also portrayed by size is regularly broad, including fields of science as different as surface science, normal science, sub-nuclear science, semiconductor physical science, essentialness storage, engineering, micro fabrication, and sub-nuclear engineering. The related investigation and applications are comparably extraordinary, running from enlargements of standard contraption physical science to new philosophies [1].

Erythrina variegata , seems to be a propagating subtropical and tropical forest evergreen tree, renowned as such a decorative because of its noticeable red petals. Throughout India, this was among the most utilized forage forest plant foods used for the feedstuffs for tiny ruminants[2]. It has been used as a hedgerow and windbreak. It is also a flashy, broad, deciduous forest legume that really can grows to a height of 18- 25 m.Various parts of *E. Variegata* have utilized in conventional medication as a nervine narcotic, febrifuge, hostile to asthmatic and antiepileptic. It is also used for the treatment of certain sicknesses like seizure, fever, aggravation, bacterial disease, sleep deprivation, helminthiasis, hack, cuts, and wounds. It fixes the feminine issue of females and also it will increase sperm count and production in males. The leaves of this

plant had colossal restorative worth it neutralize the microbial attack and fabricate the invulnerability of our body[3].

Due to increased population and outbreak of multiple microbial disease, the underground people are suffering a lot due to the high costly synthetic medicine. To offer cost effective treatment without any side effects this nanotechnology become an amazing platform for researches and pharmacological sectors to drug discovery from the source of nature[4]. In our study, we synthesis the silver nanoparticles by reducing the fresh leaves of *Erythrina variegate* and evaluated their antimicrobial and antifungal activity [5].

MATERIAL AND METHODS

Chemical reagents

Solution(Silver nitrate), solvents and basal media are utilized for this evaluation. *Staphylococcus aureus, Staphylococcus heamolytics,* and *Bacillus cereus (*Gram positive bacteria) and *Enterobacter cloacae* and *Bacillus subtilis* (Gram negative bacteria) are used for antibacterial study and *Aspergillusniger*and *Aspergillus flavus* are used for antifungal activity.

Collection of plant

The leaves of Kalyanamurungai are collected from different areas of thiruvannamalai district which are taxonomically identified. The collected leaves are washed completely a few times with deionized water. Then the dried leaves are prepared into fine powder.

Plant Extract Preparation

A 5 g of leaves have been gauged, boiled to 100 ml deionized water upto 5 min as well as the concentrates have been distilled via Whatman filter paper No. 1. The separated filtrate was put away in cooler at 4º C. This concentrates were utilized as lowering and also stabilizing agent.

Biosynthesis of Silver Nanoparticles (AgNPs)

Roughly 50 grams of dried plant matter has been steeped along with 250 mil of 80% ethanol at ambient temperature with constant stirring up to 48 hours; the whole method has been performed twice, utilizing freshly prepared solvent each time. Applying Whatman No. 1 membrane filter the sample was filtered as well as condensed to dryness using only a rotary evaporator at 65°C up to 3 hours.

Obtained filtrate has been deposited inside a 250 mL Erlenmeyer flask as well as stocked at 4ºC in an airtight container for future use. Except if in any case referenced, plant extract 1 mL of had been added to 19 mL alcohol solvent of 1.0 mM silver nitrate for AgNPs development. This solution was incubated at ambient temperature up to 24 h. This same sample had been removed occasionally as well as broke down for amalgamation of AgNPs utilizing UV-Vis spectrophotometer which was sorted out in Figure 1.

CHARACTERIZATION OF SILVER NANO PARTICLES:

UV-visible spectroscopic study of synthesized AgNPs

The silver nanoparticles that are synthesized as in the form of colloidal solution that is observed by Ultra violet-Visible spectrophotometer. Because of this special reflection properties of silver nanoparticles, the enormous number of data about the phenomenon condition of silver nanoparticles are acquired by spectral analysis, otherworldly possession of silver nanoparticles are also studied. The absorption spectra of supernatants are taken between 300 to 600 nanometre, using a UV–visible spectrophotometer. Also, UV-Visible spectroscopy gives a system to screen how the nanoparticles change after some time. [6].

Fourier Transform-Infrared analysis of synthesized AgNP:

FTIR spectroscopic spectrum have been executed for finding the potential biomolecules in leaves extract of *Erythrina variegata* that really are essential for a lowering of silver nanoparticles. A fabricated silver nanoparticle mixture had been homogenized by centrifugation at 6000 rpm for 30 minutes, and then discard the supernatant. The pellet would have been mixed in distilled water but also homogenized more to particles with in colloidal matrix. A powder specimen had been retrieved through dehydrating the cleansed pellets in a drying oven for 2 hours [7].

SEM AND TEM

SEM is the strategy to assess the surface morphology of nanoparticles where high energy electron bar was utilized to examine the outside of the example. The electron pillar and constituent molecules in the example connect with one another delivering signals containing the auxiliary data of the nanoparticles that are noteworthy to their properties and functionalities. The components present in the EV-AgNP‘s were observed using SEM.The size, shape and morphology of the nanoparticles were determined using transmission electron microscope[8].

Antibacterial potentiality of AgNps using *Erythrina variegate*

Examined the antimicrobial properties of the produced AgNPs toward some clinical isolates Gram positive pathogens like Staphylococcus haemolyticus (MG744417), Staphylococcus aureus(MH431700) , Bacillus

cereus(MH393374) and Gram negative bacteria like Enterobacter cloacae(MH553000), Bacillus subtilis was undertaken by analysing the standard well diffusion procedures (Mubarakali et al2011).New growth medium are prepared a day before was spread on the sterilized plates of Muller Hinton Agar (MHA) . S terilized cork borer has been used to make wells of 8 mm diameter in the MHA plates [10]. The wells are filled with different concentration 200µl, 150µl, 100µl, 50µl of AgNPs solution and antibiotic (Ciprofloxacin) has been referred as positive control. The MHA plates were then incubated for 28 hours at 37 °C [12]. Around the well were impregnated with AgNPs are measured for inhibition zone to evaluate the antibacterial potency of the produced AgNPs [13].

Antifungal activity of AgNps using *Erythrina variegate*

The antifungal potency of the synthesized AgNPs against *Aspergillus niger* and *Aspergillus flavus* are studied by disc diffusion method. The fresh culture are prepared in overnight were spread on mulliton agar plate[14]. The wells were loaded with different concentration 200µl/, 150µl/, 100µl/, 50µl of AgNPs solution and antibiotic (Ciprofloxacin) was used as positive control. . The plates are incubated at 37 °C for 28 hour. The impregnated wellof AgNPs were measured and the percentage of zones of inhibition were calculated to determine the antifungal activity of the synthesized AgNPs[15].

RESULT AND DISCUSSION

Visible observation:

After adding ***Erythrina variegate,*** aqueous silver ions are converted to silver nanoparticles was shown in figure 1. Also noted that the color of the solution changed from yellow to bright yellow and then to dark brown after 24 to 48 h of the reaction, that determine the development of silver nanoparticles .

UV – Vis Spectroscopy and visual analysis:

The UV-Vis spectroscopy result of the obtained solution exhibit a characteristic absorbance peak at 450nm after 2 hours was shown in figure 2. As the exceeds of reactiontime, there is the random shift in the absorbance peak from 450 to 460 nm. The increase in reaction time is directly proportional to the intensity of the absorption peak which was measured. The γmax values in the 400-500nm absorbance peak are only for the surface plasmon band of AgNPs[9].

Fourier-transform infrared spectroscopy

Using FTIR spectroscopy, substituent groups of the biomolecules had been evaluated which would be accountable for limiting and stabilizing the nanoparticles. Ranges at 3190cm-1, 2926cm-1, and 2926cm-1 had been assigned to the O–H stretch carboxylic acids as well as the C-H extend of alkane and alkyls, including both. Peaks at 781cm-1, 841cm-1,and 1636cm-1 had been connected to the C–Cl stretch and also the CH stretch of aromatic compounds, alkyl halides, and amines. The FTIR observations including purified EVAgNPs noted excitation peaks at bands at 3395cm-1, 3273cm-1,3190 cm-1, 3047cm-1, and 2957cm-1, which have been precise only for O-H stretch H bonded, O-H stretch free of alcoholic group [10,11]. The N-H, C-O, C-F,C-H, and C-H elongating pulses of aldehydes, amines, aromatic compounds, carboxylic acids, alkyl halides, alkynes and ether had been allocated to a peaks at 1636cm-1, 1366 cm-1, 1483cm-1, 886 cm-1, and 3047cm-1as shown in figure 3.

Scanning electron microscope

The structure as well as density of the silver nanoparticles were also investigated utilizing SEM and EDX-SEM pictures at various amplifications. EDX-SEM examination portrays a clusters nonuniformly conveyed AgNPs with a level of total and that are spherical and ununiformed shapes shown in figures 4. The synthetic pattern of the orchestrated AgNPs had been assessed utilizing EDX-SEM. A EDX example of every AgNPs exhibits massive electric field at 3 keV. The occurrence of highs prior to actually 5 keV indicate the existence of an unadulterated silver metal particle. The example additionally shows tops associating with the coupling energies of carbon and oxygen. Unmistakably demonstrates the effect of combined nanoparticles.

Transmission electron microscope

The structure and shape of the biosynthesized erythrina variegate silver nano particles are further observed through by TEM. The transmission electron microscope shows elliptical and spherical shape of nanoparticals clearly between 50 to 100 nm. They are dispersed randomly, but some of the particles are overlap between small and big particles. The image of the TEM clearly shown in figure 5

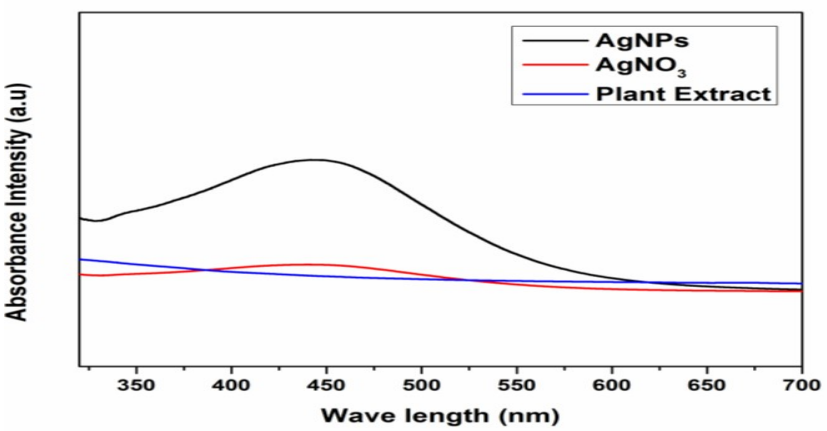
Antimicrobial Activity

The antimicrobial potential of EV-AgNPs at different concentration(50-200µl) was determined against microorganisms that, namely, *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterobacter cloacae*, *Bacillus subtilis.* The sensitivity or resistibility of the previously mentioned microorganisms against EVAgNPs, as well as the antibiotic influence (Ciprofloxacin) had been calculated by monitoring the inhibitory activity by measuring zone of Inhibiting.

EV AgNps exhibit moderate antimicrobial properties against the above mentioned isolated strains (as Figure 6 and 7, showed) with Staphylococcus haemolyticus and Bacillus cereus (Gram positive bacteria) showing the highest susceptibility compare to *Staphylococcus aureus*. *Enterobacter cloacae* and *Bacillus subtilis* (Gram negative bacteria) both determine much more resistivity at concentrations ranging from 50- 200 µg/mL of EV AgNps. The bioactive EVAgNPs showed a greater effectiveness with a 15mm inhibition zone against *Staphylococcus haemolyticus*, *Bacillus cereus*, *Enterobacter cloacae*, *Bacillus subtilis*, 14mm against *Staphylococcus aureus*, 14mm inhibition zone against *Aspergillus niger* and 13mm inhibition zone against *Aspergillus flavus* even at concentration of about 200 µg/mL, presented in Table 2, 3 and 4. EVAgNPs displayed viable antimicrobial effectiveness as compared to the positive control Ciprofloxacin.

Figure 1: Synthesis Silver Nanoparticles colour change reactions

Figure 2: Absorbance peak at 450nm



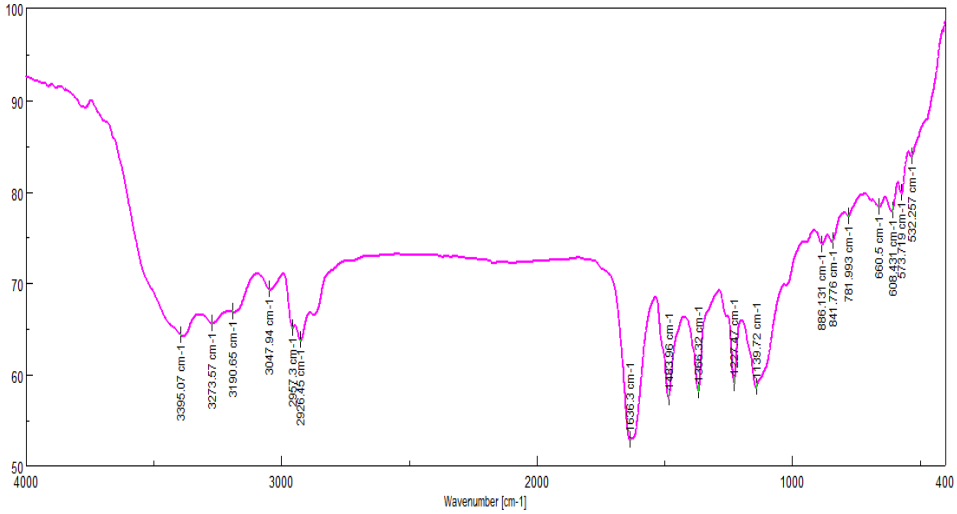


Figure 3: FTIR analysis of Ag nanoparticles synthesized using the plant extract of *Erythrina variegate*

Figure 4: Images of SEM shows the size a shape of the particles

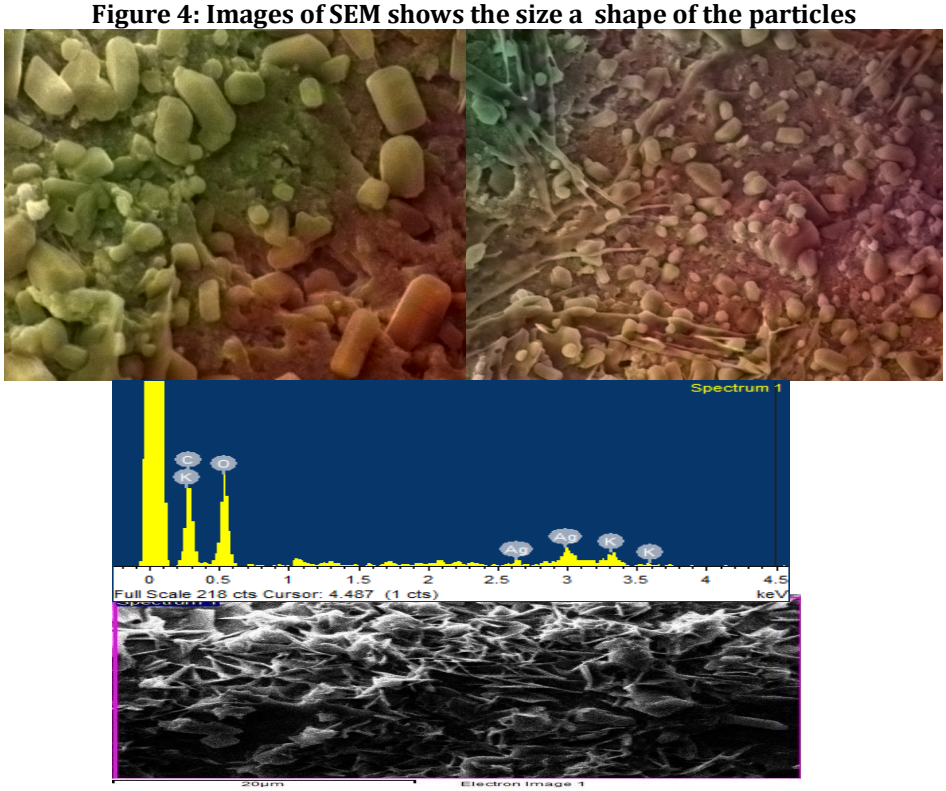


Figure 5: Transmission electron microscopy images at 50 and 100 nm

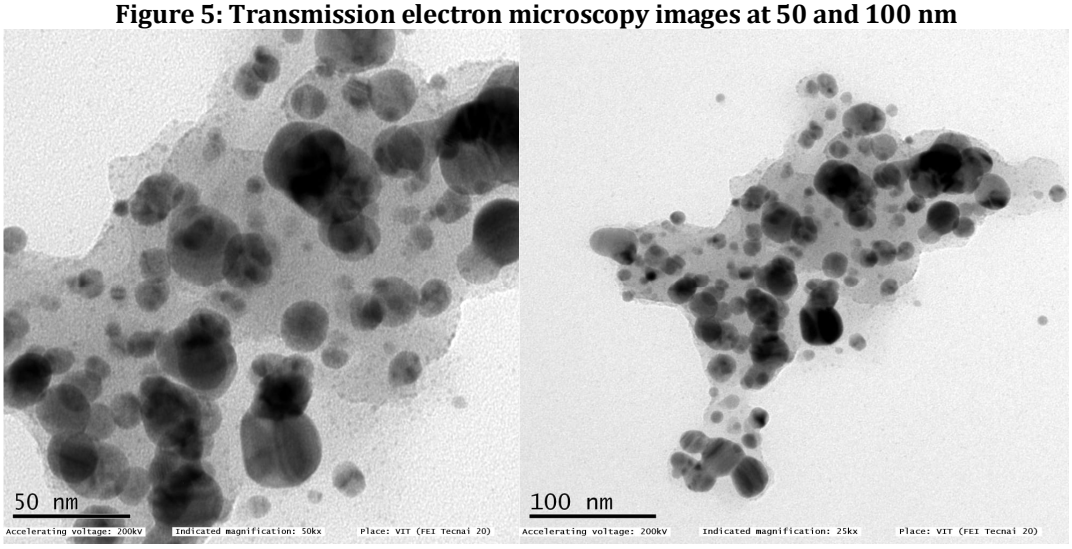


Table 1:Elements found in SEM

ELEMENTS	WEIGHT%	ATOMIC%
CK	28.37	39.89
OK	52.77	55.70
KK	5.30	2.29
Ag L	13.56	2.12

Tale 2: Zone of Inhibition of Gram positive bacteria

ZONE OF INHIBITION in mm						
S.NO	Test Organism (positive bacteria)	200µL	150µL	100µL	50µL	Standard
1	*Staphylococcus haemolyticus*	15mm	14mm	13mm	10mm	22mm
2	*Staphylococcus aureus*	14mm	13mm	-	-	23mm
3	*Bacillus cereus*	15mm	13mm	12mm	11mm	24mm

Figure 6: Antibacterial activity of EV-AgNPs against A. *Staphylococcus haemolyticus* B. *Staphylococcus aureus* C. *Bacillus cereus* D. *Enterobacter cloacae* E. *Bacillus subtilis*

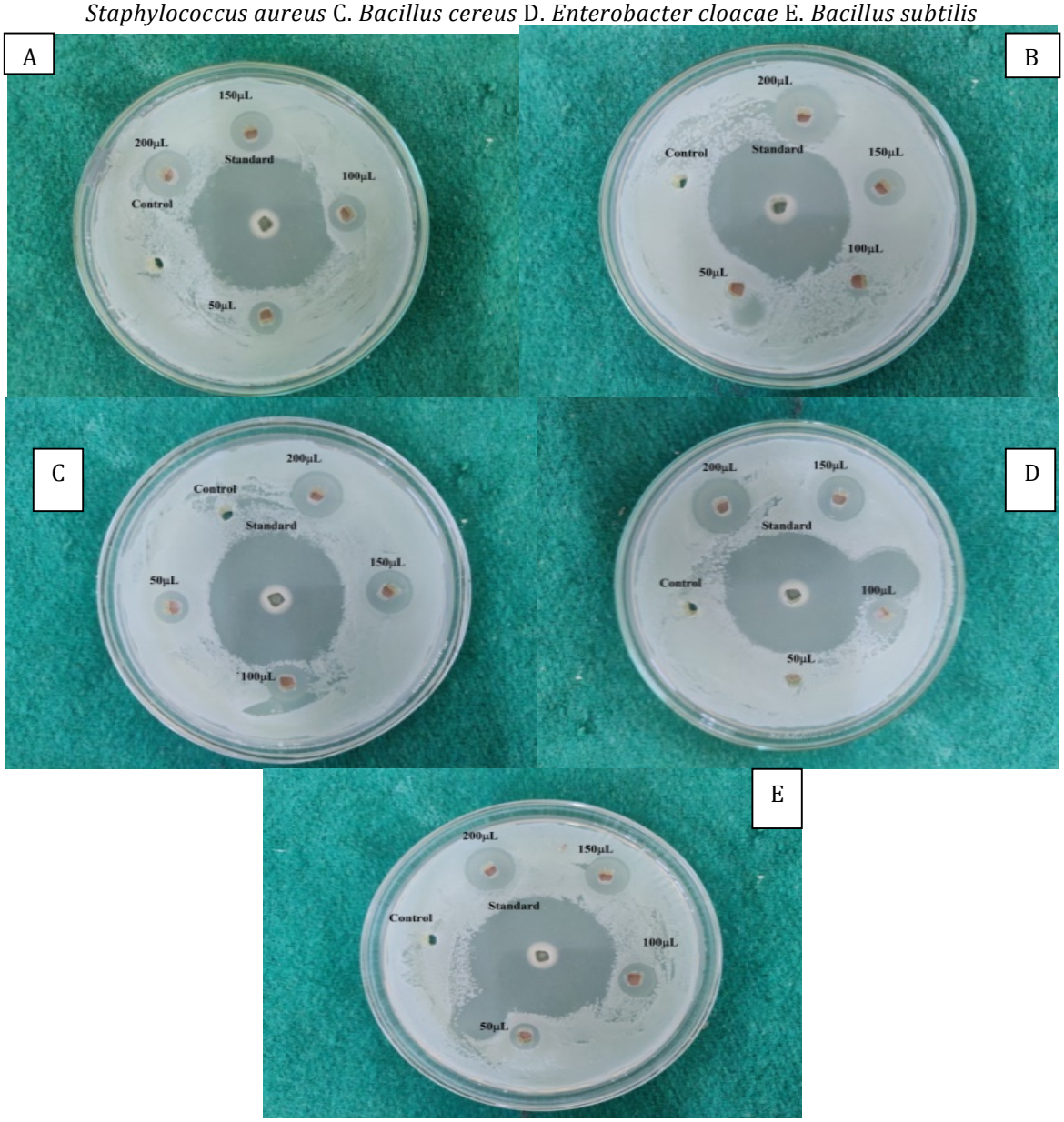


Figure 7: Antifungal activity EV-AgNPsA. *Aspergillus niger* B. *Aspergillus flavus*

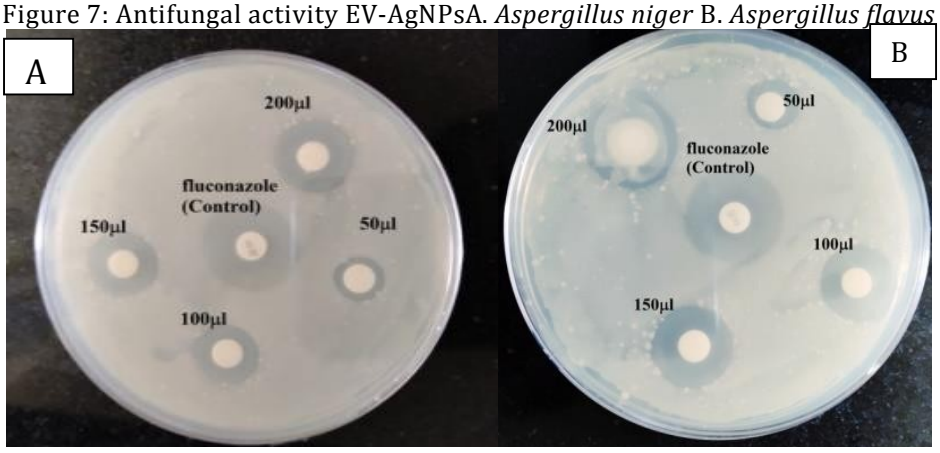


Table 3:Zone of inhibition of Gram negative bacteria

ZONE OF INHIBITION in mm						
S.NO	**Test Organism (negative bacteria)**	**200µL**	**150µL**	**100µL**	**50µL**	**Standard**
1	***Enterobacter cloacae***	**15mm**	**14mm**	**13mm**	**-**	**22mm**
2	***Bacillus subtilis***	**15mm**	**12mm**	**10mm**	**-**	**23mm**

Table 4:Zone of Inhibition of fungi

ZONE OF INHIBITION in mm						
S.NO	**Test Organism**	**200µL**	**150µL**	**100µL**	**50µL**	**Standard**
1	***Aspergillus niger***	**14mm**	**13mm**	**13mm**	**9mm**	**20mm**
2	***Aspergillus flavus***	**13mm**	**12mm**	**10mm**	**10mm**	**19mm**

CONCLUSION

From the aqueous extract of *Erythrina variegata* the silver nanoparticles are produced. The silver nanoparticles are identified by the colour change from yellow to brown that was studied by the UV Vis spectroscopy. FTIR spectral measurements were finded the potential biomolecules and EDX-SEM examination portrays a clusters no uniformly conveyed AgNPs, it shows high discharge energy at 3 keV. In 5 keV shows the presence of a unadulterated silver metal particle. The transmission electron microscope shows elliptical and spherical shape of nanoparticals clearly between 50 to 100 nm. The biosynthesized E-AgNPs shows a synergetic antimicrobial potential against Gram positive bacteria like (Staphylococcus haemolyticus, Bacillus cereus, Staphylococcus aureus), Gram negative bacteria (Enterobacter cloacae and *Bacillus subtilis*) and antifungal property (*Aspergillus niger* and *Aspergillus flavus*). The green synthesized silver nanoparticles have some predictable application in the field of medicine for effective antimicrobial potential and drug discovery in pharmaceutical sector.

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