Green Synthesis of Silver Nanoparticles Using E. Stenoclada & E. Milli Plant Extract and Its Antimicrobial Activities

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***Abstract*—The green synthesis of silver nanoparticles using the xerophytic plant extracts showing various characteristics. In the present study, synthesis silver nanoparticles by using plant extract, which are the reducing agents for synthesizing AgNPs. For better performance different solvent and solvent ratio were used including distilled water (DW), ethanol (E), methanol(M), ethanol and distilled watermixture (E/DW) (1:1, v/v) and M/DW (1:1, v/v). various important parameters used such as solvent ratio, precursor concentration of AgNO3 solution to plant extract during the study. AgNps characterization done by using U.V spectrophotometry at 520nm. Along with that antibacterial and antifungal assay also carried out, for antibacterial E. coli (gram -ve) and S.aureus (gram +ve) strains were used, likewise antifungal strains were A. alternata and A. niger were used to observed the zone of inhibition against the green synthesis of nanoparticles.**

***Index Terms*—AgNPs, Solvent and solvent ratio, U.V Spectroscopy and Antimicrobial activity.**

I. INTRODUCTION

Nanotechnology hasemerged asoneof the largest and most attractive areas for research, offering unique features and extensive applications in various sectors such as agriculture, food, and biomedicine (Erci, F.; Cakir-Koc et.al. 2018). Nanoparticles of noble metals, such as silver, gold, platinum, copper, zinc, titanium, and magnesium, have gained considerable attention for biomedical applications due to their multifunctional theragnostic abilities (Behboodi, S. et.al.2019). The green synthesis method makes the mass production of nanoparticles safer and less expensive (Haajira Beevi Habeeb Rahuman, Ranjithkumar Dhandapani, Santhoshini Narayanan, et al 2022). Photoluminescence studies of synthesised silver nanoparticles were also evaluated and it was confirmed that the protocol was simple, rapid, one step, eco-friendly, non-toxic and an alternative conventional physical/chemical method. Only 15 min were required for the conversion of silver ions into silver nanoparticles at room temperature, without the involvement of any hazardous chemical(Shakeel Ahmed, Saifullah et.al 2018).Among the nanoparticles, silver nanoparticles (AgNPs) have attracted considerable researcher's attention because of its fascinating properties, such as a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, high catalytic activity and antimicrobial activities. Reportedly, silver displays a unique role in antimicrobial, catalytic and biological systems. (Tahir Rasheed, Muhammad Bilal, et al, 2017). They are widely used as anticancer, antibacterial, and larvicidal agents due to their promising and distinct properties. (Tijjani Mustapha, NurRaihana Ithnin, et al 2023).

II. MATERIALS AND METHOD

2.1 Plant Material- The selected plants for the study i.e.,Euphorbia stenoclada,collected from Bamboo Garden Amravati and Euphorbia milli, collected from the local area of Amravati region, in February 2024 (Fig no. 1 & 2). They were identified with the help of the standard flora that is "Flora of Amravati District with Special Reference to the Distribution of Tree Species by M.A. Dhore.

2.2.

Fig . 1 *Euphorbia stenoclada*

Fig. 02: Habit of Euphorbia milli

Preparation of Euphorbia stenoclada and Euphorbia milli powder. The Euphorbia stenoclada and Euphorbia millistemsare thoroughly washed three times with double-distilled water to remove debris and dust particles. Subsequently, the leaves shade-dried at room temperature for one week until they reached a constant weight. The dried leaves are crushed into powder, followed by sieving with 0.2mm mesh. The prepared powder packed in an airtight container and stored under dry conditions for further use.

2.3 Synthesis of Es-AgNPs and Em-AgNPs

The green synthesis of Va-AgNPs was performed using leaf extracts of Euphorbia stenoclada (Fig no. 3 A) and Euphorbia milli (Fig no. 3 B) and silver nitrate (AgNO3) metal salt precursorswith the help of a magnetic stirrer equipped with a hot plate. Inwhich phytochemical components are extracted from Powder material and then used for the reduction purpose of AgNO3 (MelakuuTesfaye, YodaheGonfa et.al 2023).

A

B

Fig. 03: Fresh extract of A-Euphorbia stenoclada, B-Euphorbia milli

2.4 Solvent selection for extraction using plant leaves

The extraction of active components from Euphorbia stenoclada and Euphorbia milli stem powder carried out using different solvents, including distilled water (DW), ethanol (E), methanol(M), ethanol and distilled water mixture (E/DW) (1:1, v/v) and M/DW (1:1, v/v). Two grams of dried powder are taken and placed in a 250 mL screw-capped Erlenmeyer flask containing 100mL of solvent for the extraction process. The sample flasks are kept at 40C for 1hour under constant stirring. Then, the extract obtained using each solvent used to synthesize AgNP safter passing through WhitmanNo.1filter paper to separate there sidue. The AgNPs were synthesized by adding 50 mL of plant extract to 50 mL of a 5 mM AgNO3 solution at 50C for 1 hour under constant stirring (S.O. Aisida, et al 2019).

2.5 Characterization of synthesized Es-AgNPs and Em-AgNPs UV–vis spectroscopy.

UV–Vis molecular absorption spectroscopy is an

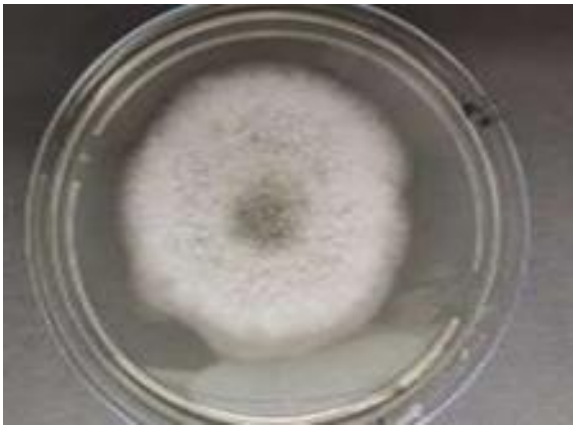
analytical technique based on the absorption of electromagnetic radiation inthe wavelength of 520nm. Greenly synthesized nano particles were characterized during a double beam UV–Vis-spectrophotometer Baseline correction of the spectrophotometer is carried out using a blank reference. The diluted EsAgNPs and Em-AgNPs are placed in aUV-cuvette and observed under a spectrophotometer. Then, the UV–Vis absorption spectra of all the samples and their characterization recorded and numerical data plotted using the observed data.

2.6 Antimicrobial Analysis

Agar disk diffusion method (simple, practical and has been well standardized) technique is used to investigate the antibacterial activities of EsAgNPs and Em-AgNPs. Antibiotic disks Paper (commercially available) with a fixed concentration was placed on the inoculated agar surface. The anti-bacterial activities of the samples were tested against two different bacteria named Staphylococcus aureus, Escherichia coli and Ciprofloxacin used as a standard and two fungal strain Alternaria alternate and Aspergillus niger and Flucanozolused as a standard, using the agar disc diffusion method. the solution was added to the petri dishes followed by incubation at 37 C for 24 h for bacteria and at 30°C for fungal strain. The inhibition zones of both the microbes were measured and recorded in millimeters.

Fig.04 E. coli & S. aureus

A



B

Fig.05 A. Aspergillusniger B. Alternaria alternate

III.RESULT & DISCUSSION

3.1 Green synthesis of Es-AgNPs and Em-AgNPs: Solvent selection for extraction

Maximize the active components by using multiple solvents instead of a single one. As a preliminary analysis, synthesis from plant extract was confirmed by observing color changes in plant extract, such as faint green and pale yellow in both samples E.stenoclada (Fig no. 6 A) and E.mili (Fig no.6 B) after reduction with AgNO3 synthesis changed the color intensity of plant extract like black, Off white, faint grey, dark brown, dark grey, off-white, red-brown, grey-black, red-brown, grey black color in E. stenoclada, and wise E. millia showing Off white. Faint grey, brown, reddish-brown, Orange, Grey black, sky blue, red-black, Grey black, and faint grey in E. milli(Table no. 1), Melakuu Tesfaye ET.AL 2023 observed color change for DW, E/DW and M/DW

based plant extract solutions from light brown to dark brown after adding them to a 5 mM AgNO3 precursor solution with a 1:1 (v/v) ratio, the formation of nanoparticles. This color change has been used as a common preliminary screening technique by various researchers [S.O.Aisida, et al2019].

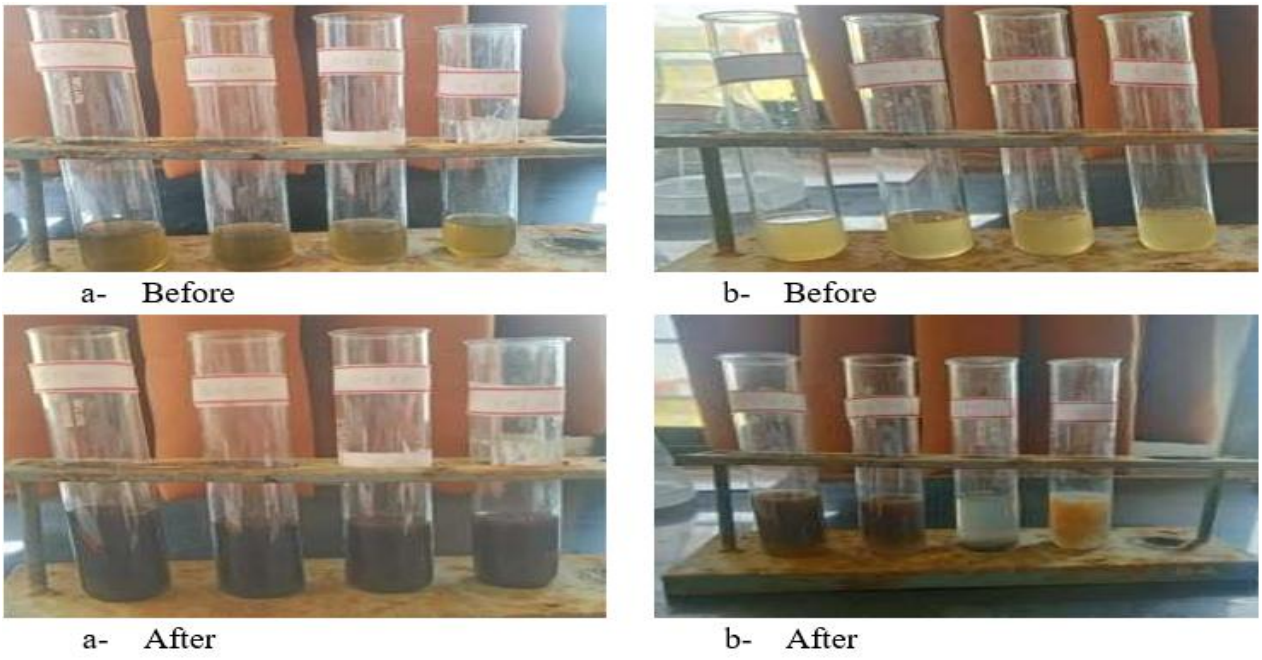


Fig. 6 Euphorbia stenoclada (A), Euphorbia milli (B) plant extract before and after adding AgNO3.

TABLENO.1 Plant Extract Color and reduced nanoparticles color in ES- AgNO3 and EM-AgNO3

Sr. No.	Solvent	Color of plant extract		Colour change after adding different concentration		
		Euphorbia stenoclada	Euphorbia Milli	Precursor Concentration	Euphorbia stenoclada	Euphorbia Milli
1.	Ethanol	Faintgreen	Faintgreen	20:80	Black	Off-white
				40:60	Black	Off white
				60:40	Black	Faint grey Faint grey
				80:20	Black	
2.	Methanol	Paleyellow	Faintgreen	20:80	Off White	Off white
				40:60	Black	Brown
				60:40	Black	Reddish-brown
				80:20	Black	Brown
3	Distilled water	Paleyellow	Faintgreen	20:80	off white dark	Orange
				40:60	brown dark grey	Grey black
				60:40	black	Grey black
				80:20		Sky blue
4	Ethanol and distilled water	Faint green	Faint green	20:80	Dark brown	Off white
				40:60	off white black	Black
				60:40	black	Grey black
				80:20		Black
5	Methanol and distilled water	Faint green	Faint green	20:80	Off white	Grey black
				40:60	red brown grey	faint grey
				60:40	black brown	grey
				80:20		faint grey

3.2 Characterization of Es-AgNPs and Em-AgNPs leaf extract

The color of the reaction mixture was observed by UV-Vi's absorption spectra and the absorbance recorded at 520 nm. Absorbance spectra vary in both plant samples according to the precursor concentration. The highest absorbance was recorded in methanol and distilled water solvents of 20:80, 40:60, 60:40 precursor concentration are 2.500 nm, 2.477nm,1.555nm, 0.933nm,0.881nm in E.stenoclada (Fig no. 7), Likewise, in E.milli (Fig no.8) the highest absorbance recorded 1.482nm and 1.451nm of methanol solvent of 20:80 and 60:40 precursor concentration. The higher absorption intensity shows that the number of AgNPs formed in the solution increases with increasing reaction time [M. Umadevi, et.al.2012, S. J. Chuchita, 2018]. Typical absorption band for silver nanoparticles was observed in the visible light region for silver nanoparticles [S. Yamamoto,et.al. 2004]. The plasmon peak and the full-width at half-maximum (FWHM) depend on the extent of colloid aggregation [S. Yamamoto,et.al. 2004]. Lowest absorbance recorded in the blank 0.00 nm to 0.002nm. (Table no. 2)

TableNo.2 Characterization of Es-NPs and Em-NPs by UVspectroscopy at 520nm.

Sr. No.	Solvent	Precursor Concentration	Absorbance	
			Euphorbia stenoclada	Euphorbia milli
1.	Ethanol	Blank	0.000 nm	O.OOOnm
		20:40	0.586nm	O.397nm
		40:60	0.323nm	O.199nm
		60:40	0.296nm	O.198nm
		80:20	0.308nm	O.222nm
2.	Methanol	Blank	0.001nm	0.000nm
		20:80	0.213nm	1.451nm
		40:60	0.534nm	1.482nm
		60:40	0.172nm	0.337nm
		80:20	0.357nm	0.863nm
3.	istilled water	Blank	0.000nm	0.000nm
		20:80	2.262nm	0.985nm
		40:60	1.647nm	0.933nm
		60:40	1.638nm	1.325nm
		80:20	0.702nm	0.881nm
4.	Ethanol and Distilled water	Blank	0.000nm	0.000nm
		20:80	0.641nm	0.269nm
		40:60	0.317nm	0.160nm
		60:40	0.307nm	0.660nm
		80:20	0.256nm	0.283nm
5.	Methanol and Distilled water	Blank	0.000nm	0.002nm
		20:80	2.500nm	0.276nm
		40:60	2.477nm	0.312nm
		60:40	1.555nm	0.238nm
		80:20	0.725nm	0.193nm

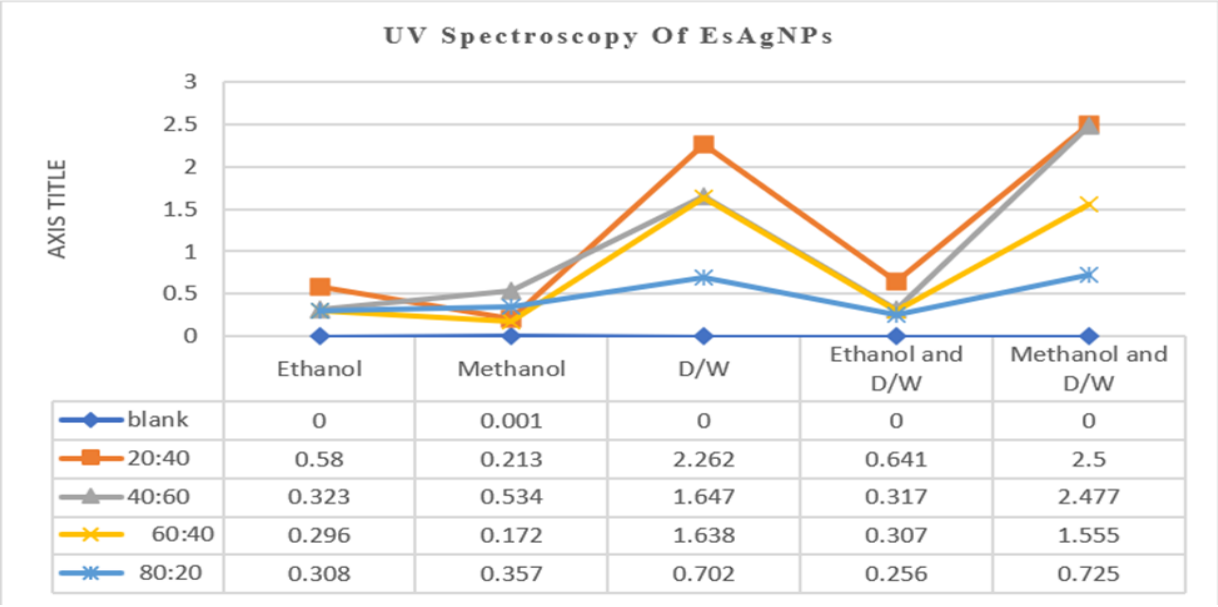


Fig.7 Uv Spectroscopy of Synthesized Es-AgNPs at 520nm with different precursor concentrations

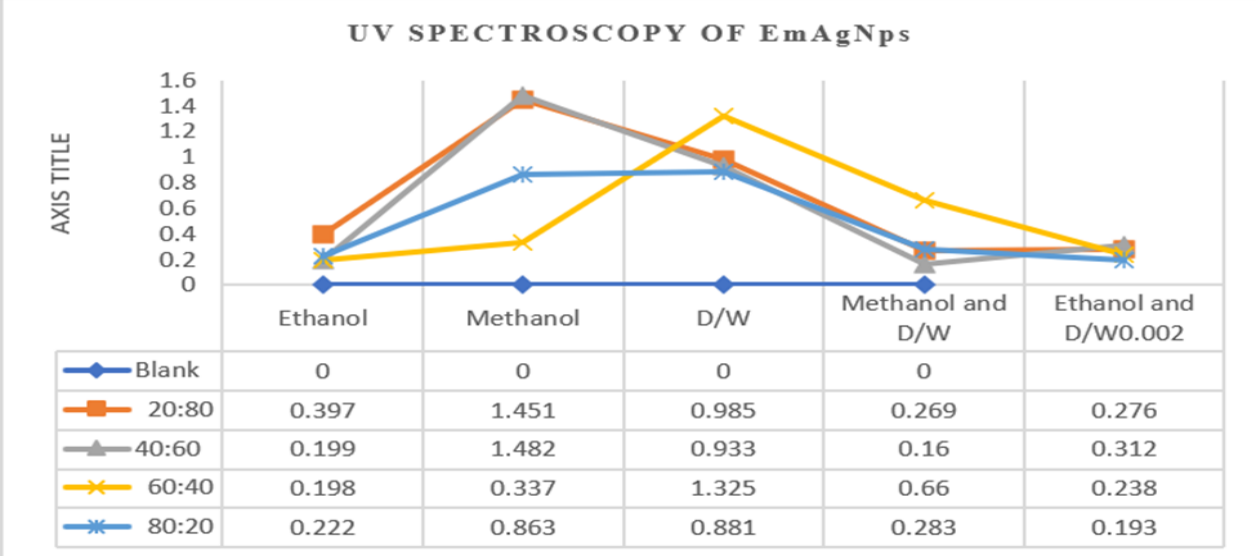


Fig 8. Uv Spectroscopy of Synthesized Es-AgNPs at 520nm with different precursor concentrations

3.3 Antimicrobial activity of Es-AgNPs and Em-AgNPs

3.3.1 Anti-bacterial analysis of Es-AgNPs and Em-AgNPs.

In present investigation highest zone of inhibition observed in standard drug (Ciprofloxacin) 3.1mm and 1.7mm in 80:20 concentration against the E. coli and 1.8mm zonation again in the standard against of E. coli in 60:40 concentration. S. aureus also showed inhibition zone in the standard Ciproflaxacine i.e 3.4mm in 80:20 precursor of Es.AgNps followed by 3.00mm and 2.9mm in precursor concentration of 20:8 both Es-AgNPs and Em-AgNPs of S.aureus. followed by both Es AgNps and EmAgNPs in all precursor concentration showed highest zonation against the E. coli and S.aureus strains (Fig no. 9 & 10), (N. Savithramma 2011) observed that the biologically synthesized silver nanoparticles, using medicinal plants, were found to be highly toxic against different pathogenic bacteria and fungi of selected species. (Table no, 3)

Table no. 3. Antibacterial Zone of inhibition against Es-AgNPs and Em- AgNPs

Sn.	Solvents	Precursor concentration	Zone of inhibition for E. coli in different concentrations		Zone of inhibition or S.aureus in different concentrations	
			Es-AgNPs	EmAgNPs	Es-AgNPs	EmAgNPs
1.	Standard (Ciproflaxin)	20:80	1.5mm	1.3mm	3cm	2.9cm
		40:60	1.1mm	1mm	1.6cm	1.9cm
		60:40	1mm	1.8mm	1.6cm	1.9cm
		80:20	1.7cm	3.1cm	3.4cm	1.7cm
2.	Ethanol	20:80	1.6cm	1.6cm	1.2cm	1cm
		40:60	1.6cm	1.4cm	1.5cm	1.3cm
		60:40	1.7cm	1.3cm	1.3cm	1.3cm
		80:20	1.7cm	1.3cm	1.9cm	1.4cm
3.	Methanol	20:80	1.6cm	2cm	1.1cm	1.9cm
		40:60	1.3cm	1.2 cm	1.8cm	1.5 cm
		60:40	1.6cm	1.4 cm	1.2cm	1.4 cm
		80:20	1.3cm	2.4 cm	1.2cm	1.2 cm
4.	Distilled	20:80	1.9cm	1.9 cm	1.5cm	1 cm
		40:60	40:60	1.5cm	1.2 cm	1.5cm
		60:40	60:40	1.2cm	1.2 cm	1.4cm

		80:20	80:20	1.7cm	1.6 cm	1.4cm
5	E: DW	20:80	2cm	1.9 cm	1.9cm	1.3 cm
		40:60	1.5cm	1 cm	1.6cm	1.4 cm
		60:40	1.4cm	1 cm	1.6cm	1.2cm
		80:20	1.3cm	1.5 cm	2cm	1.5 cm
6	M: DW	20:80	1.4cm	2 cm	1.5cm	1.5cm
		40:60	1.6cm	1.4 cm	1.5cm	1.4 cm
		60:40	1.4cm	1.9 cm	2cm	1.3 cm
		80:20	1.3cm	2 cm	2.7cm	1.1cm

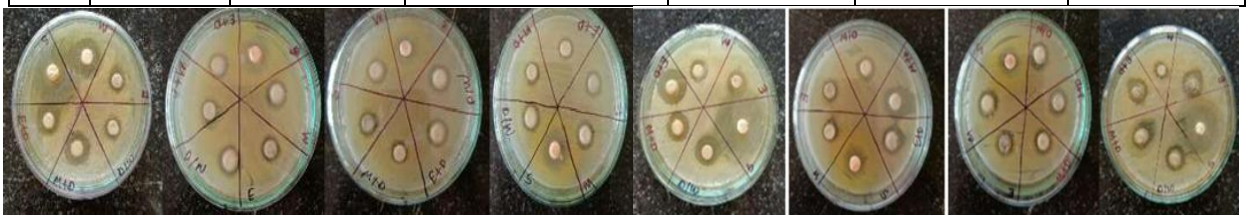
3.3.2 Antifungal analysis against Es-AgNPs and Em-AgNPs

The observed record indicates the highest zone formation in an ethanol solvent of 80:20 precursor for EmAgNPs against A.alternata i.e., 1.3 mm followed by 0.9 mm, 0.8 mm, 0.7 mm in 20:80, 40;60 and 60:40 precursor concentration against A.alternata , M:Dw also showed similar zonation in all precursor concentration (Fig no. 11 & 12), but in EsAgNps showed very less inhibition activity in all the precursor and standard (Ofloxin) also showed least inhibition activity (Melakuu Tesfaye et.al 2023). A.niger also showed highest inhibition activity against EsAgNps 1.00mm in E: Dw of 80:20 precursor concentration. A.niger showed least inhibition activity (Table no. 4)

TableNo.4 Antifungal zone of inhibition against Es-AgNPs and Em-AgNPs

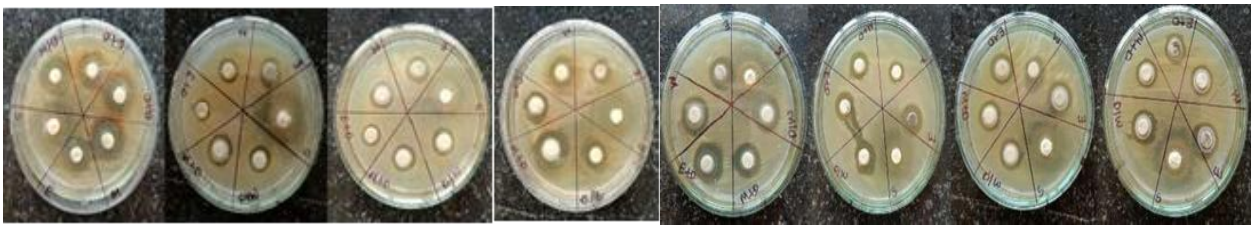
Sn.	Solvents	Precursor concentration	Zone of inhibition for A. alternate in different concentrations		Zone of inhibition for A. niger in different concentrations	
			Es-AgNPs	EmAgNPs	Es-AgNPs	EmAgNPs
1.	Standard (Ciproflaxin)	20:80	0.1mm	1cm	0mm	0.1mm
		40:60	0mm	0mm	0mm	0.7mm
		60:40	0.1mm	0.9mm	0mm	0.9mm
		80:20	0mm	0mm	0.2mm	0.8mm
2.	Ethanol	20:80	0.2mm	0.9mm	0.3mm	0.6mm
		40:60	0.4mm	0.8mm	0.5mm	0.4mm
		60:40	0.6mm	0.8mm	0.7mm	0.6mm
		80:20	0.7mm	1.3cm	0.9mm	0.6mm
3.	Methanol	20:80	0.2mm	1cm	0.3mm	0.5mm
		40:60	0.3mm	0.7mm	0.6mm	0.6mm
		60:40	0.3mm	0.9mm	0.5mm	0.8mm
		80:20	0.8mm	0.7mm	0.7mm	0.6mm
4.	Distilled water	20:80	0.3mm	1 cm	0.2mm	0.5mm
		40:60	0.2mm	0.7mm	0.8mm	0.5mm
		60:40	0.5mm	1cm	0.4mm	0.3mm
		80:20	0.6mm	0.9mm	0.8mm	0.4mm
5.	E: DW	20:80	0.1mm	1cm	0.4mm	0.2mm
		40:60	0mm	0mm	0.8mm	0.4mm
		60:40	0.1mm	0.9mm	0.6mm	0.6mm
		80:20	0mm	0mm	1cm	0.5mm
6.	M: DW	20:80	0.2mm	0.9mm	0mm	0.1mm
		40:60	0.4mm	0.8mm	0mm	0.7mm
		60:40	0.6mm	0.8mm	0mm	0.9mm

		80:20	0.7mm	1.3cm	0.2mm	0.8mm



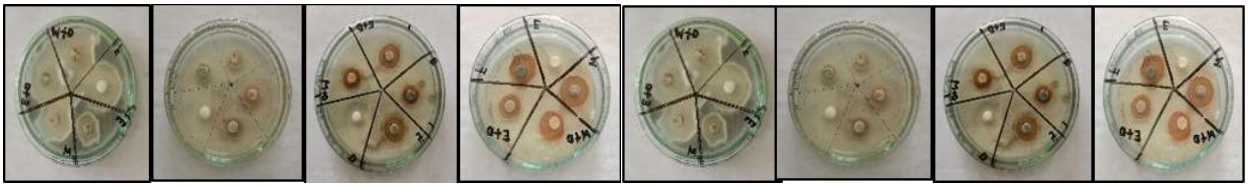
A B

Fig.9 Antimicrobial zone of inhibition for S.aureus against precursor concentrstions 20:40,40:60,60:40 80:20 Es-NPs (A) and Em-NPs(B).



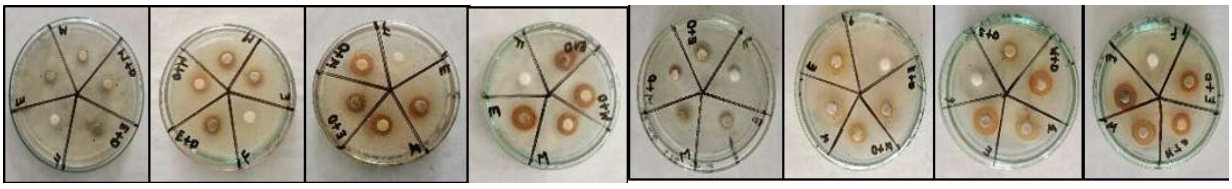
C D

Fig.10Antibacterial zone of inhibition for E. coli against precursor concentration 20:40, 40:60, 60:40, 80:20 Es-NPs(C) and Em-NPs(D)



E F

Fig.11 Antifungal zone of inhibition for A.alternata against precursor concentrations 20:40, 40:60, 60:40, 80:20 Es-NPs(E) and Em-NPs (F)



G H

Fig.12 Antifungal zone of inhibition for A.niger against precursor concentrations 20:40,40:60,60:40 80:20 Es-NPs(G) and Em-NPs (H)

IV. ACKNOWLEDGMENTS

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