METALLIC NANOPARTICLES FORMULATION AND EVALUATION OF LEAVES EXTRACT AND ISOLATED COMPOUNDS OF ACACIA CATECHU (L.) WILLD AND ACACIA AURICULIFORMIS A.CUNN

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Abstract

The aim of this research was to create a green synthesis of metallic nanoparticles using leaves extracts and isolated compounds of *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunn. Extract and isolated compounds along with excipents were used to prepare Silver nanoparticles and was evaluated for particle size, zeta potential and % EE. The synthesised AgNPs colloidal solution shown superior antibacterial activity against both Gram-positive and Gram-negative bacterial strains i.e., *Klebsiella pneumonia, Bacillus subtilis, E. coli* and *Streptococcus* sp. in testing. The diameters of the inhibition zones of AgNPs at 50 g/ml concentrations against *Klebsiella pneumonia, Bacillus subtilis, E. coli* and *Streptococcus* sp. were reported and it was found that the Silver Nanoparticles have significant antibacterial activity.

Key-words: *Acacia species,* Silver Nanoparticles, Evaluation

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DOI: 10.53555/ecb/2022.11.9.34

Introduction

Silver nanoparticles have a long history of antibacterial activities. Silver nanoparticles are actively involved in antibacterial action against a wide range of harmful bacteria and fungi that cause disease in food and drink. Various biological and green materials have been used to synthesise silver nanoparticles, including gramme positive and gramme negative bacteria such as Klebsiella pneumonia and Bacillus subtilis, Cladosporium cladosporioides, marine algae Padina tetrastromatica and Turbinaria conoides, green waste peels of banana fruits, carbohydrate molecules such as polysaccharide and disaccharide starch, sucrose, and maltose. [1-2] The plant *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunn belongs to family Fabaceae is an indigenous plant grown under wild condition in some parts of our country and was chosen for the present investigation. These plants were used in traditional system of medicine for the treatment of bacterial infection, fungal infection, diabetes, liver disorders etc. [3-4] The scanty availability of information on this plant facilitates the study on it. The attempt was made to study anti-microbial activity of extract and isolated compounds.

Material and Methods

Biosynthesis of Silver nanoparticles

$AgNO_3$ powder was dissolved in distilled water to prepare 10 mM $AgNO_3$ stock solution from which a series of 1 mM, 2 mM and 3 Mm $AgNO_3$ solutions were prepared. The AgNO3 solutions were mixed with the ethanolic extract of ACL, AAL, C1, and C2 at a ratio of 1:1 and 1:2 v/v to a volume of 50 mL in a flask. The flask was wrapped with an aluminum foil and was then heated in a water bath at 60°C for 5 hours. Furthermore, the mixture was stored in the refrigerator for the further use. [5-6]

Optimization of formulation of Silver nanoparticles

Table 1: Different formulation of silver nanoparticles using ACL (*Acacia catechu* (L.) Willd)

Formulation Code	Extract (mg)[ACL)	AgNO3 (mM)	Ratio
F1	250	1	1:1
F2	250	2	1:1
F3	250	3	1:1
F4	250	1	1:2
F5	250	2	1:2
F6	250	3	1:2

Table 2: Different formulation of Silver nanoparticles using AAL (*Acacia auriculiformis* A.Cunn

Formulation Code	Extract (mg)[AAL)	AgNO3 (mM)	Ratio
F7	250	1	1:1
F8	250	2	1:1
F9	250	3	1:1
F10	250	1	1:2
F11	250	2	1:2
F12	250	3	1:2

Table 3: Different formulation of Silver nanoparticles using C1 (Lupeol)

Formulation Code	Extract (mg)[C1)	AgNO3 (mM)	Ratio
F13	250	1	1:1
F14	250	2	1:1
F15	250	3	1:1
F16	250	1	1:2
F17	250	2	1:2
F18	250	3	1:2

Table 4: Different formulation of Silver nanoparticles using C2 (Lupenone)

Formulation Code	Extract (mg)[C2)	AgNO3 (mM)	Ratio
F19	250	1	1:1
F20	250	2	1:1
F21	250	3	1:1
F22	250	1	1:2
F23	250	2	1:2

F24	250	3	1:2

Characterization of synthesized silver nanoparticles formulations [5-6]

Microscopic observation of prepared silver nanoparticles

An optical microscope (Cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared silver nanoparticle formulation.

Percentage Yield

The prepared silver nanoparticle with a size range of 200-300nm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres[3].

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug. Entrapment efficiency was determined by dialysis method. Silver nanoparticle entrapped extract were isolated from the free drug using dialysis method. The above said formulations were filled into dialysis bags and the free drug dialyzed for 24 hr. into 50 ml of buffer pH 1.2. The absorbance of the dialysate was measured against blank buffer pH 1.2 and the absorbance of the corresponding blank was measured under the same condition. The concentration of free flavonoids could be obtained from the absorbance difference based on standard curve.

Surface charge and vesicle size

The particle size and size distribution and surface charge were obtained by Dynamic Light Scattering method (DLS) (SAIF RGPV Bhopal, Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the silver nanoparticles was based on the zeta potential that was estimated according to Helmholtz–Smoluchowsky from electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm. pH, drug content and drug release were determined for optimized formulation using standard procedure.

Antibacterial Activity of Synthesized Silver Nanoparticles [7-8]

The antibacterial activity of synthesized silver nanoparticles was performed by agar well diffusion method against pathogenic bacteria, *Klebsiella pneumonia, Bacillus subtilis, E. coli* and *Streptococcus* sp. Fresh overnight culture of each strain was swabbed uniformly onto the individuals' plates containing sterile Luria Bertani agar and 5 wells were made with the diameter of 6 mm. Then 25 μL of purified silver nanoparticles, extract, and silver nitrate solution were poured into each well and commercial antibiotic discs are placed as control and incubate for 24 h at 37^0C. After incubation the different levels of zonation formed around the well and it was measured. This experiment was repeated for three times.

Results and Discussion

The synthesized silver nanoparticles containing leaves extracts and isolated compounds of *Acacia catechu* (L.) Willd and *Acacia auriculiformis* A.Cunn. was characterized. The results were presented in table 5. From the results obtained it was showed that formulation code F4, F10, F16 and F22 of each extract and isolated compounds of both the selected plant showed maximum % entrapment efficiency, therefore these formulation were selected to determine the drug content and pH. The in vitro drug release was given in table 7. The results were mentioned in table 6. The results of anti-microbial activity were given in table 8.

Table 5: Characterization of Silver Nanoparticles

Formulation Code	% Yield	% Entrapment efficiency	Average Particle size (nm)	Zeta Potential (mV)
F1	54.10±0.04	64.89	97.93	-30.28
F2	60.42±0.11	72.23	131.22	-31.27
F3	63.73±0.18	70.41	119.10	-33.48
F4	**79.58±0.12**	**86.29**	**126.25**	**- 35.5**
F5	71.81±0.10	84.22	146.89	-32.20
F6	64.10±0.11	71.11	106.04	-30.20

F7	68.29±0.09	77.10	109.11	-31.22
F8	70.42±0.08	78.32	119.23	-33.32
F9	73.21±0.12	69.09	106.17	-32.10
F10	**74.26±0.18**	**79.20**	**121.22**	**-34.39**
F11	70.27±0.12	72.32	110.29	-32.27
F12	69.86±0.24	74.40	120.62	-31.44
F13	72.88±0.04	80.29	131.54	-30.39
F14	75.14±0.22	79.22	129.29	-31.30
F15	76.31±0.02	78.24	126.40	-32.29
F16	82.39±0.05	**86.20**	**146.43**	**-33.11**
F17	80.16±0.06	78.10	136.29	-32.04
F18	79.08±0.14	76.19	129.28	-31.11
F19	71.11±0.12	80.42	113.90	-30.18
F20	73.26±0.18	79.32	122.72	-34.39
F21	76.39±0.10	76.29	129.88	-34.18
F22	81.62±0.26	**85.18**	**135.43**	**-34.78**
F23	80.28±0.18	80.28	130.29	-32.02
F24	79.02±0.11	79.39	129.49	-31.19

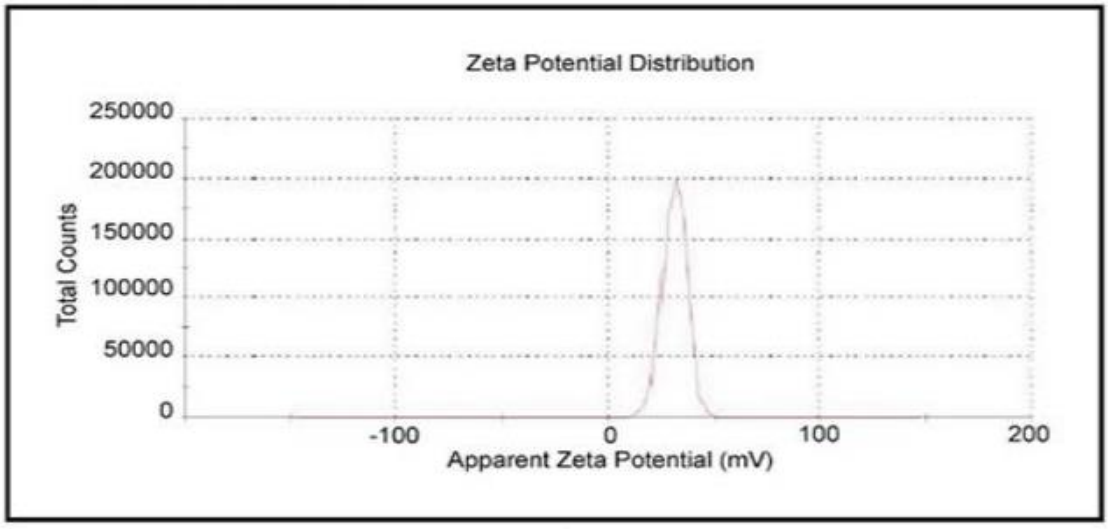

Fig. 1: Zeta Potential of F4

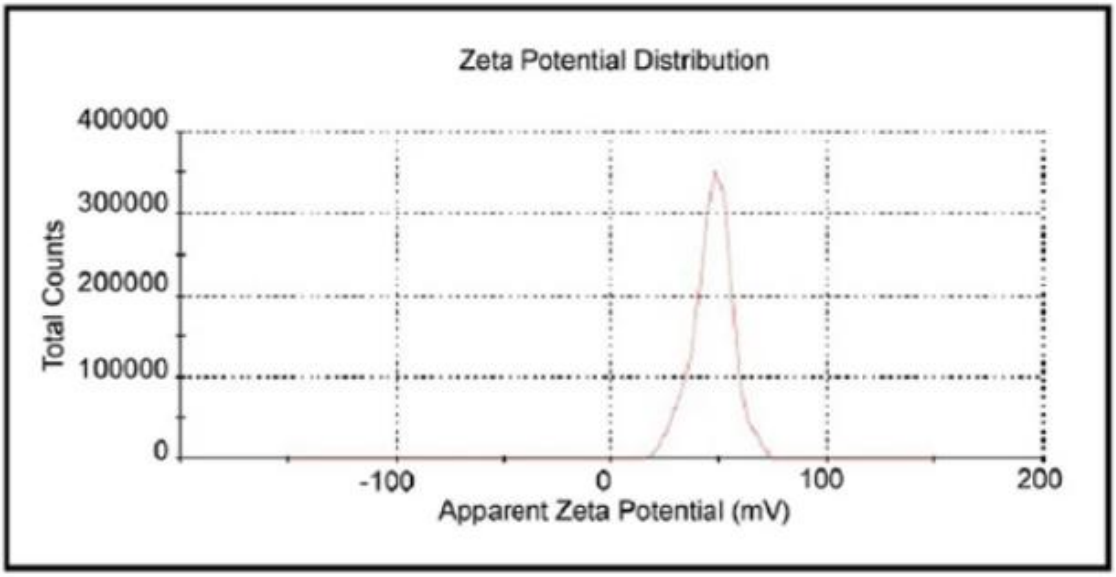

Fig. 2: Zeta Potential of F10

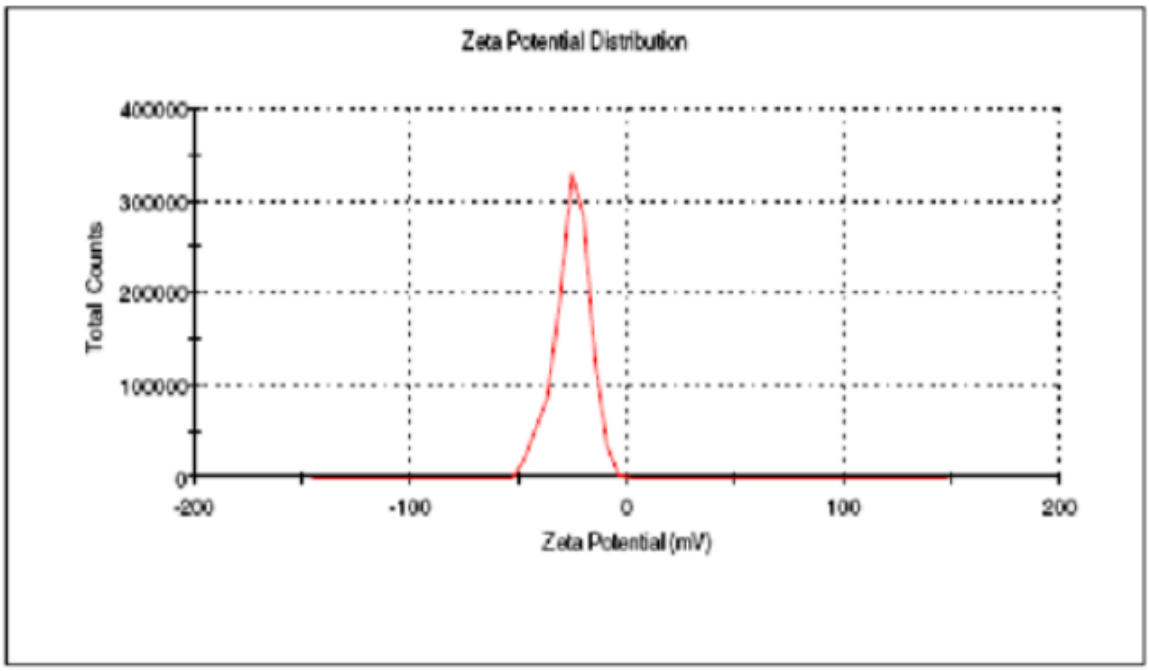

Fig. 3: Zeta Potential of F16

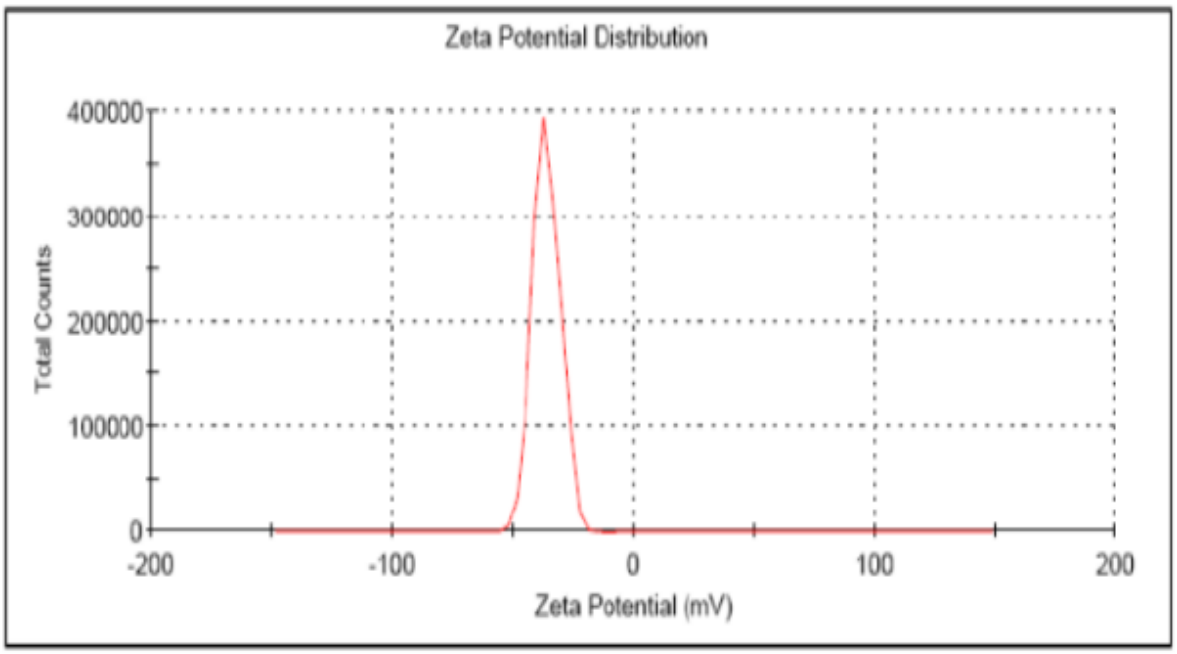

Fig. 4: Zeta Potential of F22

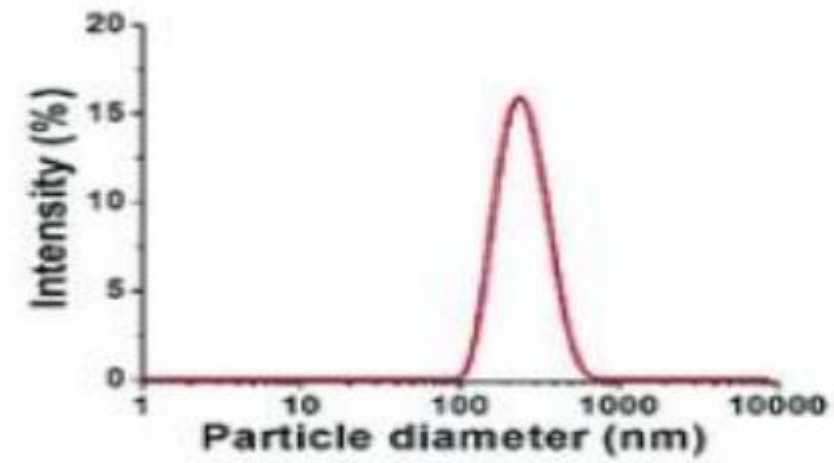

Fig. 5: PDI of F4

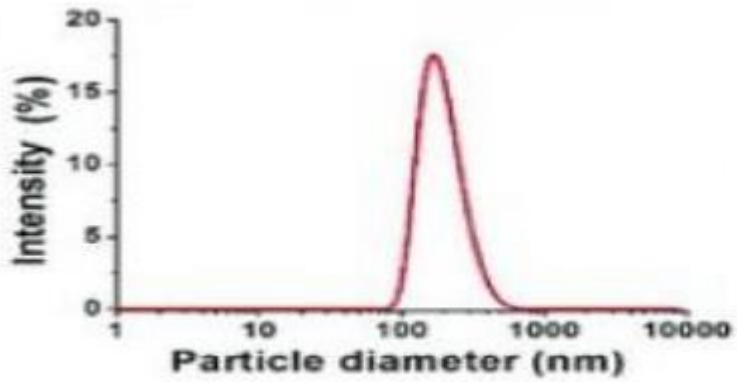

Fig. 6: PDI of F11

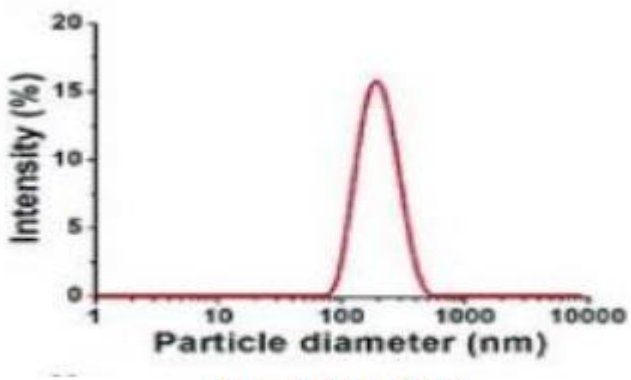

Fig. 7: PDI of F16

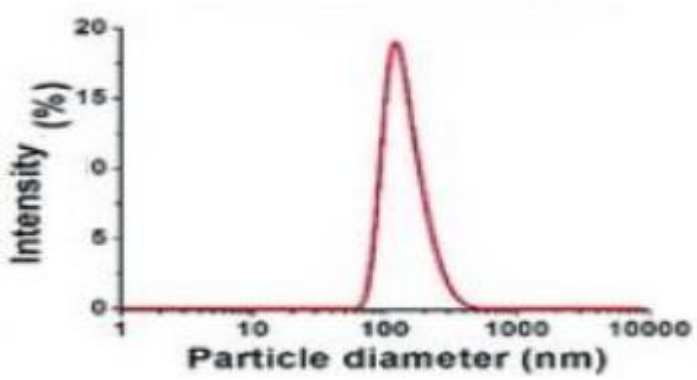

Fig. 8: PDI of F12

Table 6: Evaluation parameters of Silver Nanoparticles

Formulation Code	**pH**	**Drug content (%)**
F4	5.8	76.29
F10	6.9	78.10
F16	6.9	88.20
F22	6.8	86.16

Table 7: In-vitro drug release of Silver Nanoparticles

Time (hr)	**% Drug release**			
	F4	**F10**	**F16**	**F22**
0	0	0	0	0
10	21.5	22.6	25.2	24.5
20	34.3	35.1	38.4	36.1
30	44.2	41.5	48.8	44.9
40	55.8	54.3	59.5	56.3
50	63.4	58.9	62.3	60.0
60	68.6	68.3	77.9	74.4
70	74.8	76.7	84.1	82.0

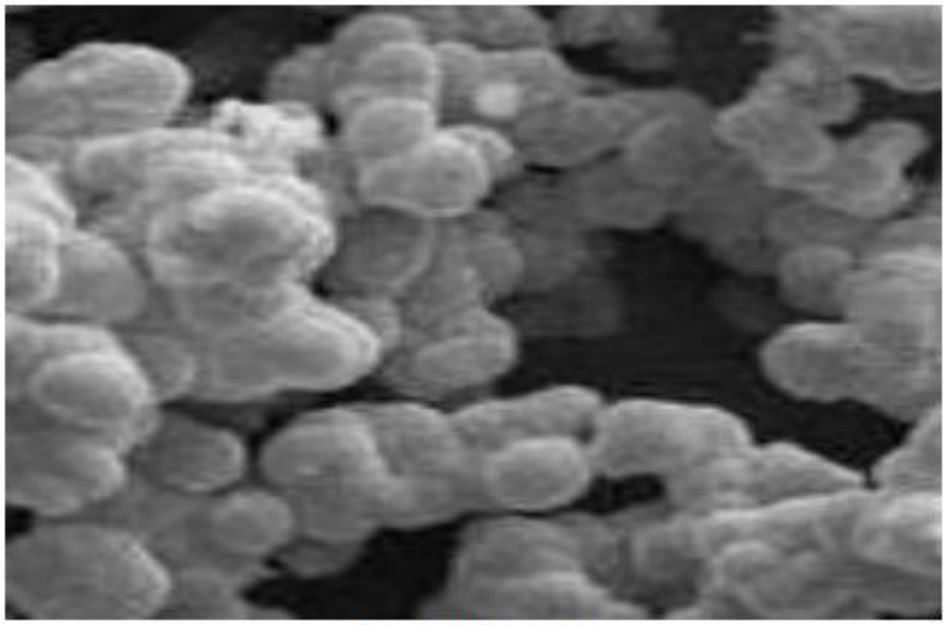

Fig. 9: SEM of F4

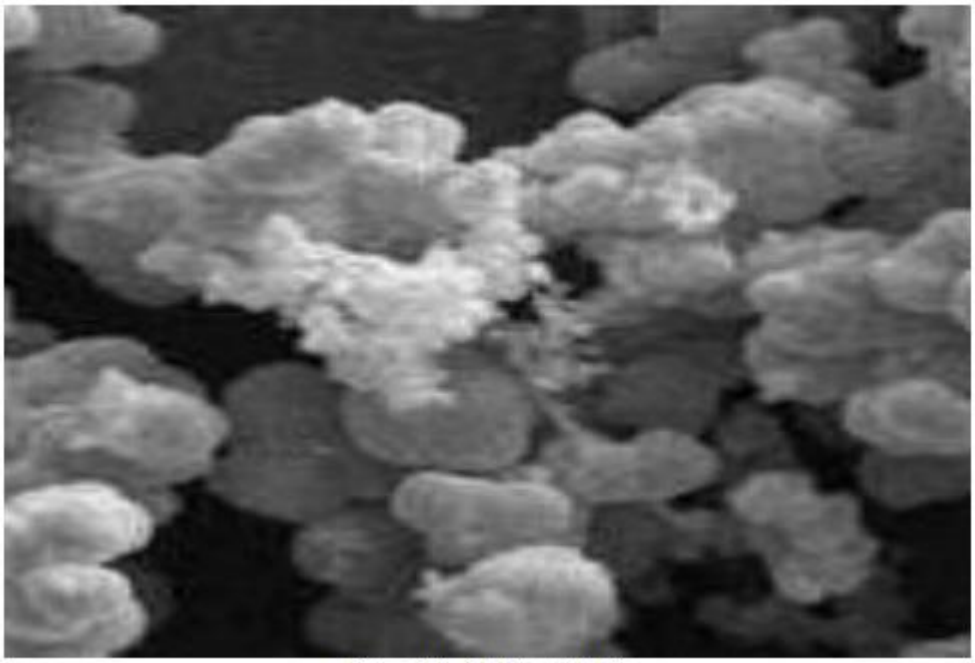

Fig. 10: SEM of F10

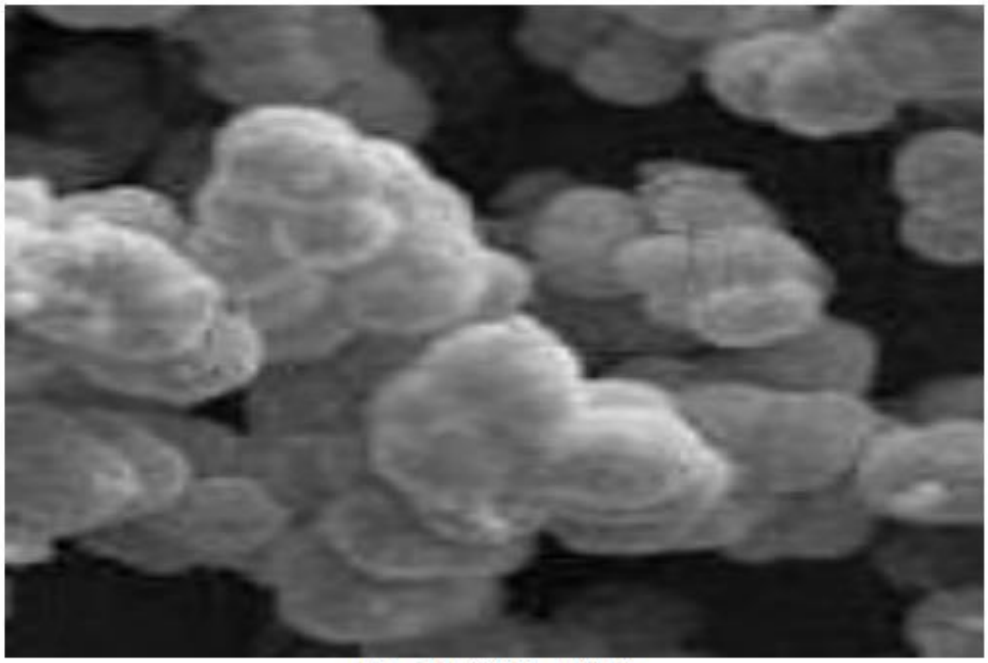

Fig. 11: SEM of F16

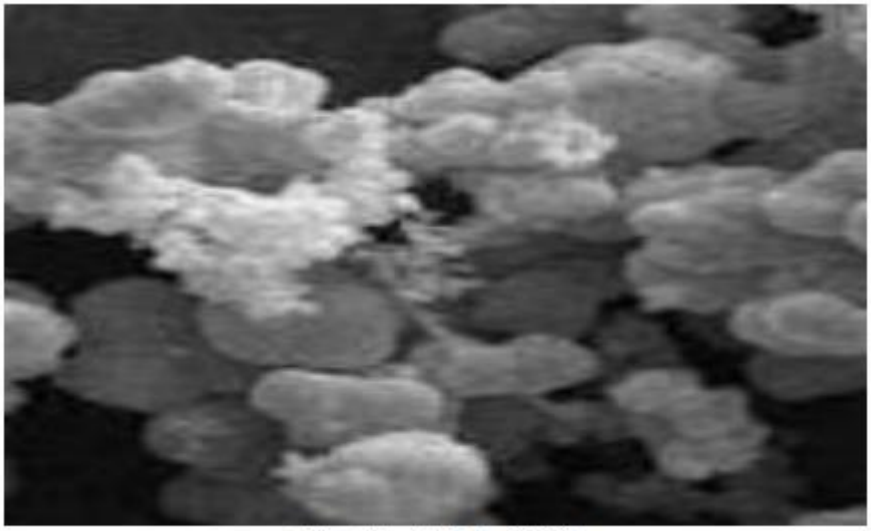

Fig. 12: SEM of F22

Table 8: ZOI of prepared Silver Nanoparticles

Antibacterial agents	**Zone inhibition (mm in diameter)**			
	B. subtilis	***Streptococcus* sp.**	***E. coli***	***K. pneumonia***
Silver nitrate solution	9.9±0.22	12.86±0.17	11.29±0.22	12.18±0.62
Commercial antibiotic disc	10.8±0.16	13.39±0.32	10.18±0.06	12.87±0.03
F4	13.89±0.11	15.26±0.11	13.48±0.02	15.26±0.11
F10	13.63±0.23	16.02±0.08	13.86±0.11	15.38±0.15
F16	11.10±0.04	15.19±0.12	12.82±0.03	13.25±0.04
F22	11.48±0.16	15.20±0.06	12.96±0.02	14.10±0.02

Conclusion

According to the findings, silver nanoparticles made from leaves extract and isolated with extract and AgNO3 in a 1:2 ratio. Formulation F22 demonstrated superior efficacy in terms of yield and % EE, hence it was chosen as the best formulation. F22 antibacterial activity was further tested using four different bacterial strains, and it was discovered that the generated silver nanoparticles have strong antibacterial activity. Because of its remarkable efficiency as an antibacterial agent, this green synthesized nanoparticle could be exploited in the medical field to treat human ailments.

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