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Green synthesis of silver nanoparticles from the aqueous extract of the seed endosperm of *Syzygium cumini*; Characterization and antibacterial activity

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- **Novelty and Highlights:**

1 –The silver nanoparticles were synthesized biologically using the extracts of the seed of the plant *Syzygium cumini* (Jambolan).

2 – The stable silver nanoparticles were characterized by means of UV/Visible Spectrophotometer, Potentiometer and Scanning Electron Microscope.

3 –The prepared green particles were analysed antibacterial effect against bacterial pathogens.

- **Graphical Abstract:**

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Green synthesis of silver nanoparticles from the aqueous extract of the seed endosperm of *Syzygium cumini*; Characterization and antibacterial activity

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Abstract: In the present study silver nanoparticles were synthesized biologically using the extracts of the seed of the plant *Syzygium cumini* (Jambolan). Phytochemical components in the extracts of the Jambolan seed were recorded. The stable silver nanoparticles were characterized by means of UV/Visible Spectrophotometer, Potentiometer and Scanning Electron Microscope. The nanoparticles exhibited maximum absorbance at 412nm in the UV spectra. SEM micrographs revealed that the synthesized nanoparticles were in the range 20-50nm. The antibacterial activity of the extracts of Jambolan seed increased in the presence of lower concentration of green synthesized silver nanoparticles of the eight bacterial pathogens used for antibacterial assay except *Eschericha coli* all the others like *Klebsiella pneumonia*, *Vibrio parahaemolyticus*, *Plesiomonas shigelloides*, *Shigella spp*, *Streptococcus aureus*, *Pseudomonas aeruginosa* and *Vibro alginolyticus.*

Keywords: *Silver nanoparticles, Seed endosperm extract, antibacterial*

Introduction

Metal nanoparticles had been reported to contain a good biomedical potential [1, 2] instead of chemicals, plant extracts are employed to synthesis nanomaterials. Green synthesis of nanoparticles is eco-friendly and nanoparticles synthesized using green technology had been reported contain a good medicinal value [3-6]. Green synthesised nanoparticles offers numerous medial applications in pharmaceuticals, water purification, textile, drug designing, drug targeting, molecular imaging etc., [7-9]. In the present study green synthesis of silver nanoparticles by using the seed extract of the plant (Jambolan seeds) *Syzygium cumini* was carried out. The nanoparticles were characterized by UV/Vis double beam spectrophotometer, potentiometer and Scanning Electron Microscope (SEM). Antibacterial activity of the raw extracts synthesis silver nanoparticles were evaluated against eight bacterial pathogens.

Experimental

Materials *Syzygium cumini* seed endosperm, silver nitrate ($AgNO_3$), Double distilled water, Whatman No 1 filter paper.

Extraction of seed extracts the seeds the tree *Syzygium cumini* were collected from Sri Paramakalyani College campus, Alwarkurichi, Tirunelveli (Dist), Tamilnadu, India in the month of July 2013. The collected seeds were washed with Double distilled water and the outer cuticle was removed. The seeds were dried at room temperature and powdered. Five grams of seeds endosperm powder was boiled with 100ml of DD water at 90^{o}C for 30 minutes. The aqueous extracts were then filtered by using Whatman No 1 filter paper. After filtration the extracts was used for further studies.

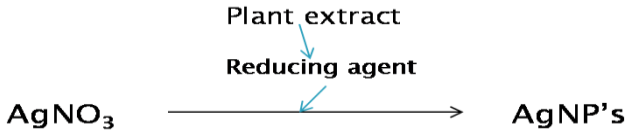
Preliminary phytochemical study of plant aqueous extract The extracts were tested for phytochemical composition like carbohydrate, protein, amino acid, alkaloid, flavonoid, tannins, saponins, terpenoids, aromatic acids, phenolic compounds, xanthoprotein, reducing sugar and triterpenoids using standard procedure [10, 11].

Preparation of $AgNO_3$ solution $AgNO_3$ was purchased from Merk Limited, Baroda. To 0.17grams of $AgNO_3$, 1000ml DD H_2O was added and $AgNO_3$ was dissolved.

This was stored in a clean brown bottle and keep away from light.

Synthesis of AgNPs ninety millilitres of $AgNO_3$ solution was added to 10ml of plant extract and stored in a room temperature without any disruption. The colour of the solution was changed in to dark reddish brown indicating the AgNPs formation.

Fig 1: preparation of AgNPs using *Syzygium cumini* seed endosperm aqueous extract.



Characterization the synthesized AgNPs were analysed using potentiometer, UV/Vis Spectrophotometer and SEM. The potentiometer study was carried out using $AgNO_3$ solution and required volume of plant extract in a beaker and time to time electric and magnetic field (EMF) was noted. The rate of absorption of UV/Vis region was analysed by spectrophotometer in the range of 200 to 800nm. Scanning electron micrographs have a large depth field yielding a characteristic three dimensional appearance useful for understanding the surface structure of a sample.

Antibacterial Assay the antibacterial activity of the raw extract of Jambolan seed and green synthesized NPs was tested against eight bacterial isolates using agar well diffusion method [12]. The zone of inhibition was measured and expressed in millimetres. Antibacterial activity was recorded if the zone of inhibition was greater than 8 mm [13]. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9mm zone was considered as inactive; 9-12mm as partially active; while13-18mm as active and >18mm as very active [14, 15].

Results and discussion

Phytochemicals study the preliminary phytochemical screening of the aqueous extracts of the seed is summerized in the Table 1. The aqueous extracts contained carbohydrates, proteins, alkaloid, tannins and reducing sugars. The phytochemicals in the seed act as a reducing agent or capping agents in the nanoparticles synthesis process [3]. These phytochemicals oxidized and to reacted with the silver nitrate solution to form silver nanoparticles.

S.No	Test for	seed aqueous extract
1	Carbohydrate	+
2	Protein	+
3	Amino acid	-
4	Alkaloids	+
5	Flavonoids	-
6	Terpenoids	-
7	Tannins	+
8	Saponin	-
9	Aromatic acids	-
10	Phenolic acid	-
11	Xanthoproteins	-
12	Reducing sugar	+
13	Triterpenoids	-
14	Phlobatinins	-

Table 1: Phytochemicals screening of Jambolan fruit seed endosperm aqueous extract.

Potentiometer Study the $AgNO_3$ solution was reduced to form AgNPs using the jambolan seed extracts. The seed extract act as a reducing agent and capping agent. The kinetics of $AgNO_3$ solution was measured by potentiometer. The time taken of Ag^+ ions reduced from $AgNO_3$ solution was measured by EMF (volts) rating (Table 2). The Ag^+ ions reduction time was 55 minutes because the EMF ranges were constant after 55 minutes.

S.No	Time taken (minutes)	EMF (volts)
1	0	318
2	5	315
3	10	314
4	15	311
5	20	305
6	25	280
7	30	276
8	35	272
9	40	269
10	45	265
11	50	199
12	55	198
13	60	198
14	65	198

Table 2: Kinetic of Ag NP's synthesized using *Syzygium cumini* seed endosperm aqueous extract.

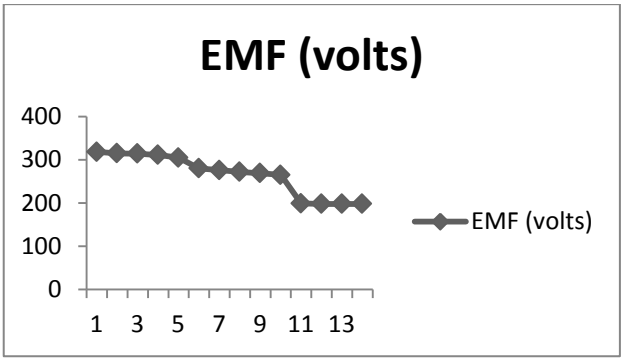


Fig 2: Potentiometer analysis of nanoparticles preparation.

UV/Visible Spectral, SEM and TEM characterisations the colour of the prepared AgNPs was dark reddish brown. The absorption strongly depends on the size of nanoparticles and chemical surroundings. The plant extracts had acted as a reducing agent and capping agent. It converts $AgNO_3$ solution to form the AgNPs by reduction method. The initial reaction of $AgNO_3$ and plant extract within 10 minutes the absorption peak was obtained in the visible region at 453nm. After 50 minutes the absorption peak was abtained from the visible region at 412nm. The variables of the absorption spectra are different in different time duration. As the $AgNO_3$ solution was completely reduced by the plant extract the absorption range got slowly reduced and the absorption peak was obtained in the visible region at 412nm (Fig 3).

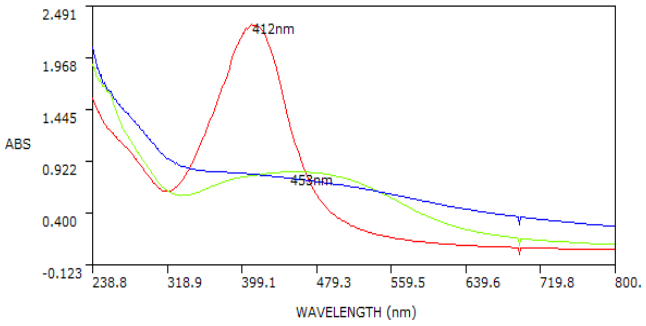


Fig 3: UV-Spectrum analysis of silver nanoparticles.

Scanning Electron Microscope images confirmed the formation of AgNPs. The surface characters were analysed by SEM images. These images confirmed the nanoparticles structure was spherical in shape and the size range 20nm to 50nm (Fig 4).

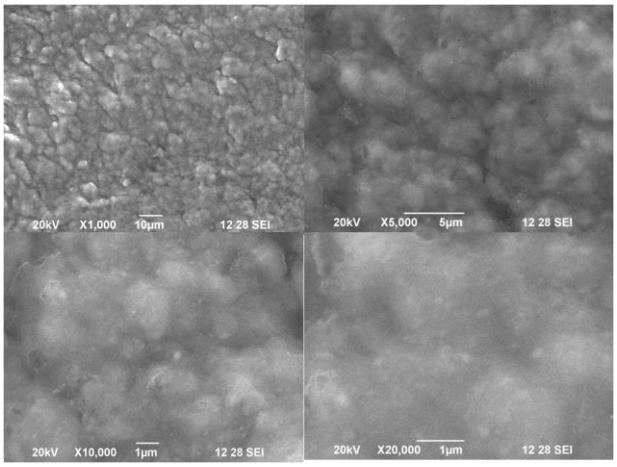


Fig 4: SEM analysis of AgNPs.

Transmission Electron Microscope images confirmed the size of the green synthesized silver nanoparticles are 65.13nm (Fig 5).

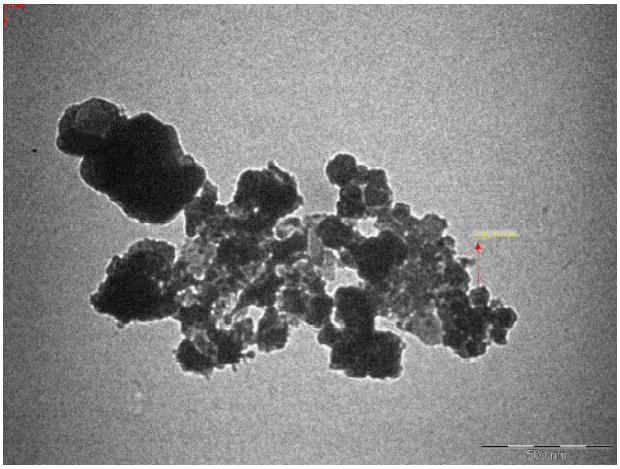


Fig. 5: TEM image of AgNPs.

Antibacterial assay the green synthesized NPs had inhibited the microbial growth (Table 3). These NPs was highly inhibiting growth of *S. aureus* (18mm). The AgNPs had rapture the cell wall of the microorganisms.

S.No	Microorganisms	Zone of Inhibition (mm)				
		25µl	50µl	75µl	100µl	S
1	*Klebsiella pneumoniae*	10	11	13	15	10
2	*Vibrio parahaemolyticus*	11	11	14	15	13
3	*Plesiomonas shigelloides*	11	15	16	16	12
4	*Shigella spp*	12	13	14	16	14
5	*E.coli*	0	0	0	12	11
6	*Streptococcus aureus*	11	15	17	18	12

7	*Pseudomonas aeruginosa*	10	12	14	15	12
8	*Vibrio alginolyticus*	12	13	15	17	14

Table 3: Antimicrobial activity of green synthesized Silver NPs using *Syzygium cumini*:

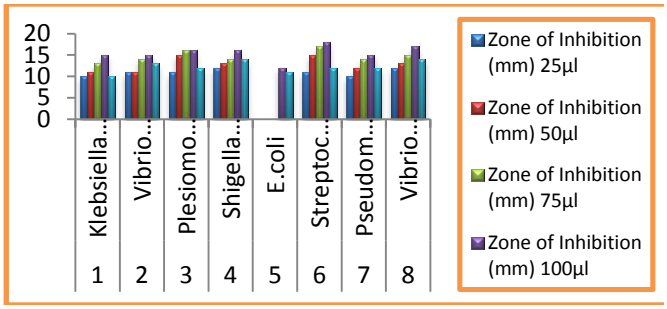


Fig 5: Antimicrobial activity of silver nanoparticles

Conclusions

The use of green synthesized silver nanoparticles in medicine is safe and it is an effective biosensitive material. The biologically synthesized AgNPs are very safe and eco-friendly when compared to the chemically synthesized nanoparticles. The green synthesized silver nanoparticles have good inhibitory activity against *Klebsiella pneumonia, Vibrio parahaemolyticus, Plesiomonas shigelloides, Shigella spp, Streptococcus aureus, Pseudomonas aeruginosa* and *Vibro alginolyticus.* As the microorganisms are developing resistance to many antibiotics, the silver nanoparticles can be used to prepare novel antibiotics also.

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