

## ANTIMICROBIAL ACTIVITY AGAINST GANGRENE USING SILVER NANOPARTICLES SYNTHESIZED FROM *ANDROGRAPHIS PANICULATA*

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### ABSTRACT

Nanoparticles have been widely used in the field of nanoscience and medicine. In the present investigation, we synthesized nanoparticles using medicinally important plant *Andrographis paniculata* with 1mM and 3mM silver nitrate (AgNO<sub>3</sub>) solution in the ratio 1:9 (Leaf: Silver nitrate Solution) were used to synthesize the silver nanoparticle. They act as reducing agent. To synthesis the silver nanoparticle. The synthesized silver nanoparticles were characterized using UV-VIS spectroscopy, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and Fourier Transform Infra-red

Spectroscopy (FTIR). The appearance of dark brown colour and the UV absorbance for *Andrographis paniculata* at 430 nm confirms the synthesis of silver nanoparticles. The aim of the study is to determine the antibacterial and antifungal activity of the synthesized silver nanoparticles from the leaf extract of *Andrographis paniculata*. The most predominantly found microbial species in gangrene are *Pseudomonas auringenosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Clostridium perferingens*, *Enterococcus faecalis*, *Candida albicans*, *Staphylo coccus*. The important outcome of the study will be the development of value added products from above mentioned medicinal plants of India for biological and nanotechnology based industries.

**KEYWORDS:** *Andrographis paniculata*, *Clostridium perferingens*, *Candida albicans*, silver nanoparticle, Gangrene.

## INTRODUCTION

Gangrene is a disease of the skin and soft tissues, sometimes internal tissues and organs that results in tissue death (necrosis). It is usually external, at the extremities, but can also affect internal tissues. Causes are most commonly chronic illness, such as a severe complication of diabetes, or acute, from certain types of injury, for example, Dry gangrene is caused by chronic illness, while wet gangrene including gas gangrene is usually an acute form involving bacterial infection and caused by injury. Wet gangrene can result from chronic disease if the dry gangrene becomes infected. Surgical complication can lead to internal gangrene, which presents signs of toxic shock. The infection caused by the microorganism such as *Clostridium species* and *Candida albicans* is most common in the gangrene affected patients. The present treatments for gangrene were Surgery (i.e. Amputation), Maggot therapy, treating infection with antibiotic medication, hyperbaric oxygen therapy. Recently nanotechnology has induced great scientific advancement in the field of medical research and technology. The synthesis of metal and semiconductor nanoparticles is a vast area of research due to its potential applications which was implemented in the development of novel technologies. The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Nanoparticle show completely new or improved properties, such as size, distribution and morphology of the particles. In the case of silver particles, the nano crystals are usually grown from Ag<sup>+</sup> solutions. The silver ions come from a salt like silver nitrate (AgNO<sub>3</sub>). The ions are first reduced to atoms by means of a reducing agent. The obtained atoms then nucleate in small clusters that grow into particles. Depending on the availability of atoms, which in turn depends on the silver salt to reducing agent concentration ratio the size and shape of the nanoparticles can be controlled.

Novel applications of nanoparticles and nano materials are emerging rapidly on various fields. But, synthesis of nanoparticles using plant extracts is the most adopted method of green, eco-friendly and also has a special advantage that the plants are widely distributed, easily available, much safer to handle and act as a source of several metabolites silver nanoparticles possess an excellent biocompatibility, low toxicity, silver has antibacterial activity in lesser concentration which rules out cytotoxicity. The major advantage of using plant extracts for synthesizing silver nanoparticle is that they are easily available, safe and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions and are quicker than microbes in the synthesis. One of the medicinal plant were

selected for study as nanodrug. The plant used for the treating the gangrene infection is *Andrographis paniculata*.

*Andrographis paniculata* (Acanthaceae) is commonly known as “King of Bitters”. The vernacular name of the species in various languages are Bengali – Kalmegh, English – The Creat, Gujarathi – Kariyatu; Hindi – Kariyat; Kalpanath; Kannada – Nilaberu; Sanskrit – Kalmegha; Tamil – Nilavembu; Telugu – Nilavembu. It is an herb and known for its hepatoprotective, antihepatitis B and anticancer activity. The active compounds in leaves are andrographolides, kalmeghin and andrographin. The leaves contain the highest amount of andrographolide, the most medicinally active phytochemical in the plant, while the seeds contain the lowest. *Andrographis paniculata* is being used mainly for treating fever, liver disease, diabetes, snake bite, antibiotic, antiviral, antimicrobial, anti inflammatory, anticancer, anti-HIV and anti-allergic.

*Andrographis paniculata* grows erect to a height of 30– 110 cm in moist, shady places. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance-shaped leaves have hairless blades measuring up to 8 centimeters long by 2.5 wide. The small flowers are borne in spreading racemes. It contains many yellow brown seed.

## MATERIALS AND METHODS

### PREPARATION OF SAMPLE

#### Collection of Samples

The fresh leaves of *Andrographis paniculata* (Nilavembu) were collected from the region of Mamandur (near Chengalpet).

#### Aqueous extract Preparation

##### Materials

Fresh leaves of *Andrographis paniculata*, distilled water, beaker, mortar and pestle, Whatman filter paper No. 1.

##### Methodology

The fresh leaves *Andrographis paniculata* was washed with distilled water and were dried for few minutes in room temperature. 20g of fresh leaves of *Andrographis paniculata* were weighed, grinded completely using mortar and pestle and the aqueous extract was filtered

using the whatman filter paper No.1. The aqueous extracts of *Andrographis paniculata* were used for the further synthesis process.

### Boiled extract Preparation

#### Materials

Fresh leaves of *Andrographis paniculata*, distilled water, beaker, microwave oven (LG), whatman filter paper No. 1.

#### Methodology

The fresh leaves and *Andrographis paniculata* was washed with distilled water and the leaves were allowed for air drying for few minutes in room temperature. 10g of *Andrographis paniculata* leaves were weighed chopped into fine pieces and were boiled in the microwave oven for 5 minutes at 40°C with 20 ml of distilled water. The boiled extract was filtered using the whatman filter paper No.1. The boiled extracts of *Andrographis paniculata* were used for the further synthesis process.

### PREPARATION OF SILVER NITRATE SOLUTION

$$\text{Molarity} = \frac{\text{Molecular Weight} \times \text{Required Molarity} \times \text{Required Volume}}{1000}$$

#### Materials

Silver nitrate (Qualigens), beaker, weighing balance, distilled water, measuring cylinder.

#### Methodology

1. 1mM silver nitrate solution was prepared by weighing 0.169g of nitrate and dissolved in 1000ml distilled water.
2. 0.509g silver nitrate was dissolved in 1000ml of distilled water to give a final concentration of 3mM silver nitrate solution.

### STANDARDIZATION

### SYNTHESIS OF SILVER NANOPARTICLES

#### Materials

Aqueous extract of *Andrographis paniculata*, 1mM silver nitrate solution, 3mM silver nitrate solution, conical flask, beaker.

### Methodology

To 5ml aqueous extract of *Andrographis paniculata*, 45 ml of 1mM and 3mM Silver nitrate solution was added in the ratio of 1:9. The prepared solution was exposed to sunlight for few minutes and the colour changed from green to brown indicates the formation of silver nanoparticles. The solution was left undisturbed for few minutes, for silver nanoparticles to settle down. The formation of silver nanoparticles from the plant extract was observed visually.

### Materials

Boiled extracts of *Andrographis paniculata*, 1mM silver nitrate solution, 3mM silver nitrate solution, conical flask, beaker.

### Methodology

To 5ml boiled extract of *Andrographis paniculata*, 45 ml of 1mM and 3mM silver nitrate solution was added in the ratio of 1:9 and the solution was exposed to sunlight for few minutes and colour changed from green to brown indicates the formation of silver nanoparticles. The solution was left undisturbed for few minutes, for silver nanoparticles to settle down. The silver nanoparticles were not formed in the boiled extract of *Andrographis paniculata*. **Hence the further work was preceded with the synthesis of silver nanoparticles obtained only from the aqueous extract of *Andrographis paniculata*.**

## SYNTHEIS OF SILVER NANOPARTICLES

### Synthesis of 1mM silver nanoparticles

#### Materials

Aqueous extract of *Andrographis paniculata*, 1mM silver nitrate solution, beaker, conical flask, measuring cylinder, centrifuge tubes, test tubes, centrifuge.

#### Methodology

To 5ml aqueous extract of *Andrographis paniculata*, 45 ml of 1mM Silver nitrate solution was added and exposed to sunlight for few minutes. The solution colour changed from green to brown indicates the formation of silver nanoparticles. The solution was left undisturbed for few minutes, for silver nanoparticles to settle down. The solution was transferred into the centrifuge tubes, centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the pellet (silver nanoparticles) was used for further process. The pellet is transferred to petri plates and left for drying at room temperature.

## Synthesis of 3mM silver nanoparticles

### Materials

Aqueous extract of *Andrographis paniculata*, 3mM silver nitrate solution, beaker, conical flask, measuring cylinder, centrifuge tubes, test tubes and centrifuge.

### Methodology

To 5ml aqueous extract of *Andrographis paniculata*, 45 ml of 3mM Silver nitrate solution was added and the solution is exposed to sunlight for few minutes. The solution colour changed from green to brown indicates the formation of silver nanoparticles. The solution was transferred into the centrifuge tubes, centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the pellet (silver nanoparticles) is used for further process. The pellet is transferred to petriplates and left for drying at room temperature.

## CHARACTERIZATION OF SYNTHESIZED SILVER NANOPARTICLES

### UV-VISIBLE SPECTROSCOPY

#### Materials

UV – Visible spectrophotometer (Systronics), cuvette, distilled water, tissue paper and samples.

#### Methodology

The leaves extracts of *Andrographis paniculata* were characterized by UV-Vis spectroscopy and the peak obtained between 400-700nm confirms the synthesis of silver nanoparticles.

### SEM Analysis

#### Methodology

The morphological features of synthesized silver nanoparticles from *Andrographis paniculata* leaf extracts were studied using the JOEL – FESEM at Sastra University Tanjore. A thin layer of gold is coated to the samples to increase its conductivity. Then the samples were characterized in the SEM at an accelerating voltage of 3.0 KV.

### TEM Analysis

#### Methodology

Samples were dispersed in double distilled water. A drop of thin dispersion is placed on a “Staining mat”. Carbon coated copper grid is inserted into the drop with the coated side

upwards. After about ten minutes, the grid is removed and air-dried. Then screened in JOEL JEM 100SX Transmission Electron Microscope at an accelerating voltage of 80 kV.

### FTIR Analysis

#### Methodology

The Fourier Transform Infra-Red Spectroscopy measurements were carried out to identify the possible functional groups present in the AgNPs. Perkin-Elmer spectrometer FTIR Spectrum one in the range 4000–400  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  was used. The sample was mixed with KCl procedure from Sigma. Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform Infra-Red [FTIR] for the analysis of the nanoparticles.

### Antibacterial Activity

#### Antibacterial activity of silver nanoparticles

##### Materials

Stock culture of *Pseudomonas aurigenosa*, *Klebsilla pneumonia*, *Proteus vulgaris*, *Enterococcus faecalis*, Muller Hinton Agar (MHA), peptone water, test tubes, petriplates, inoculation loop, laminar air flow chamber, distilled water, samples (1mM, 3mM *Andrographis paniculata*), antibiotic disc, bunsen burner, micropipette, cotton, micropipette tips, gel puncture, incubator (Yorco), autoclave (Yorco), conical flask, Dhona electronic balance, standard (Mc Farland).

##### Methodology

Subculturing was done from the stock culture of *Pseudomonas aurigenosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterococcus faecalis* sub culturing was done on the MHA plates. Single colony of the corresponding species were inoculated into 5ml of peptone water and incubated at 37°C for 20 minutes. After incubation the turbidity of the peptone water was compared with Mc Farland (0.5) standard and was swabbed in separate MHA plates. After swabbing in the plates immediately the wells were created using gel puncture. Samples of 1 $\mu\text{g}$  (1mM, 3mM *Andrographis paniculata*) were loaded into the wells respectively and the positive (amoxicillin) and negative (distilled water) controls were also maintained in the corresponding plates. The plates were incubated at 37°C for 24 hrs. The plates were observed for the zone of inhibition.



### Antibacterial activity of silver nitrate solution

#### Materials

Stock culture of *Pseudomonas aurigenosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterococcus faecalis*, Muller Hinton Agar (MHA), peptone water, test tubes, petriplates, inoculation loop, laminar air flow chamber, distilled water, 1mM and 3mM AgNO<sub>3</sub> solution, antibiotic disc, bunsen burner, micropipette, cotton, micropipette tips, gel puncture, incubator (Yorco), autoclave (Yorco), conical flask, standard (Mc Farland).

#### Methodology

Subculturing was from the stock culture of *Pseudomonas aurigenosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterococcus faecalis* sub culturing was done on agar plates. Single colony of the corresponding species were inoculated into 5ml of peptone water and incubated at 37°C for 20 minutes. After incubation the turbidity of the peptone water was compared with Mc Farland (0.5) standard and was swabbed in separate MHA plates. After swabbing in the plates immediately the wells were created using gel puncture. Samples of 5µl (1mM, 3mM AgNO<sub>3</sub> solution) were loaded into the wells respectively and the positive (amoxicillin) and negative (distilled water) controls were also maintained in the corresponding plates. The plates were incubated at 37°C for 24 hrs. The plates were observed for the zone of inhibition.

### Antifungal Activity

#### Antifungal activity of silver nanoparticle

#### Materials

Stock culture of *Candida albicans*, Sabouraud Dextrose Agar (SDA), peptone water, test tubes, petriplates, inoculation loop, laminar air flow chamber, distilled water, samples(1mM, 3mM *Andrographis paniculata*), antibiotic disc, bunsen burner, micropipette, cotton, micropipette tips, gel puncture, incubator (Yorco), autoclave (Yorco), conical flask, Dhona electronic balance, standard (Mc Farland).

#### Methodology

Subculturing was done from the stock culture of *Candida albicans* on SDA plates. Single colony of the corresponding species were inoculated into 5ml of peptone water and incubated at 37°C for 10 minutes. After incubation the turbidity of the peptone water was compared with Mc Farland standard (0.5) and was swabbed in separate SDA plates. After swabbing in the plates immediately the wells were created using gel puncture. Samples of 1µg (1mM, 3mM *Andrographis paniculata*) were loaded into the wells respectively and the positive



(Nystatin) and negative (Distilled water) controls were also maintained in the corresponding plates. The plates were incubated at 37°C for 24 hrs. The plates were observed for the zone of inhibition.

### Antifungal activity of silver nitrate solution

#### Materials

Stock culture of *Candida albicans*, Sabouraud Dextrose Agar (SDA), peptone water, test tubes, petriplates, inoculation loop, laminar air flow chamber, distilled water, samples (1mM, 3mM AgNO<sub>3</sub> solution), antibiotic disc, bunsen burner, micropipette, cotton, micropipette tips, gel puncture, incubator (Yorco), autoclave (Yorco), conical flask, Dhona electronic balance, standard (Mc Farland).

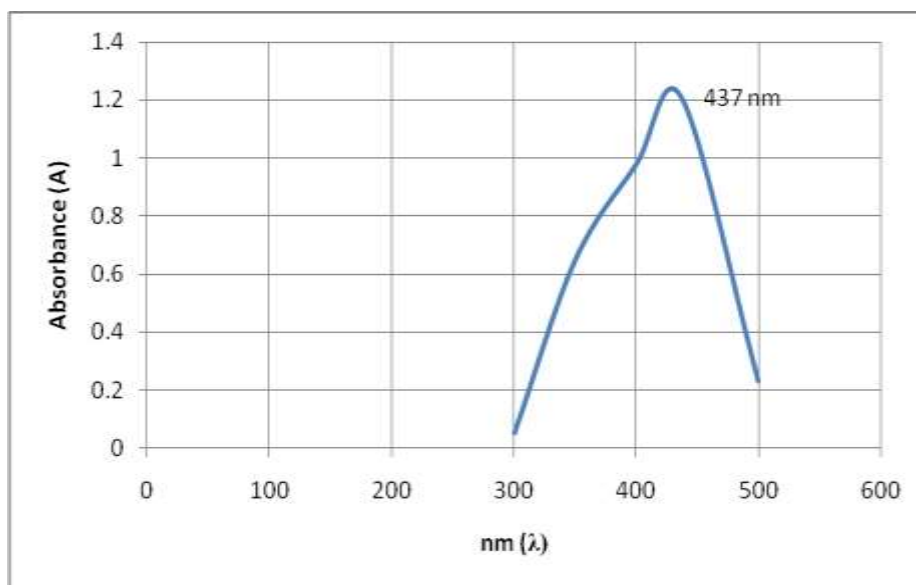
#### Methodology

Subculturing was done from the stock culture of *Candida albicans* on SDA plates. Single colony of the corresponding species were inoculated into 5ml of peptone water and incubated at 37°C for 10 minutes. After incubation the turbidity of the peptone water was compared with Mc Farland standard (0.5) and was swabbed in separate SDA plates. Using gel puncture the wells were created immediately after swabbing in the plates. Samples of 5µl (1mM, 3mM AgNO<sub>3</sub> solution) were loaded into the wells respectively and the positive (Nystatin) and negative (Distilled water) controls were also maintained in the corresponding plates. The plates were incubated at 37°C for 24 hrs. The plates were observed for the zone of inhibition.

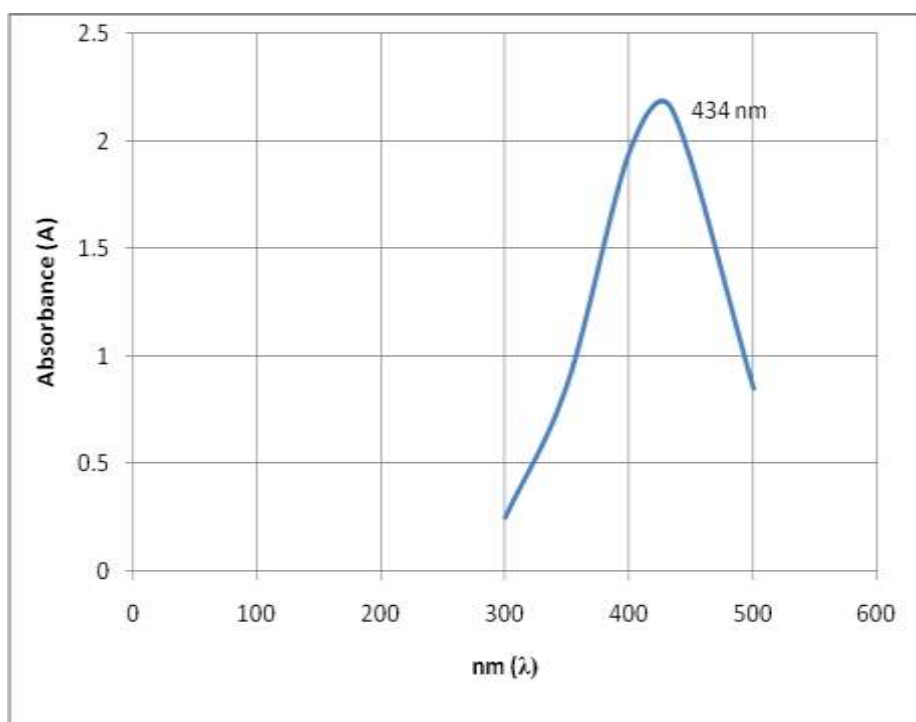
## RESULTS AND DISCUSSIONS

### UV – Visible Spectroscopy

The colour change of the leaf extract was observed, confirms the silver nanoparticles synthesis. The colour change is due to the Surface Plasmon Resonance phenomenon. The sharp bands of silver nanoparticles were observed for 1mM concentration of *Andrographis paniculata* 437 nm (Fig. 1) and for 3mM concentration 434 nm (Fig. 2) were obtained. From different literatures studies it was observed that the silver nanoparticles shows peak at around 430 nm for *Andrographis paniculata*. The peak formation in the range of 400 nm to 440 nm clearly indicates the synthesis of silver nanoparticles from the aqueous leaves extra.



**Fig. 1:** UV-Vis absorption spectra of silver nanoparticles synthesized from *Andrographis paniculata* leaves at 1mM silver nitrate solution.

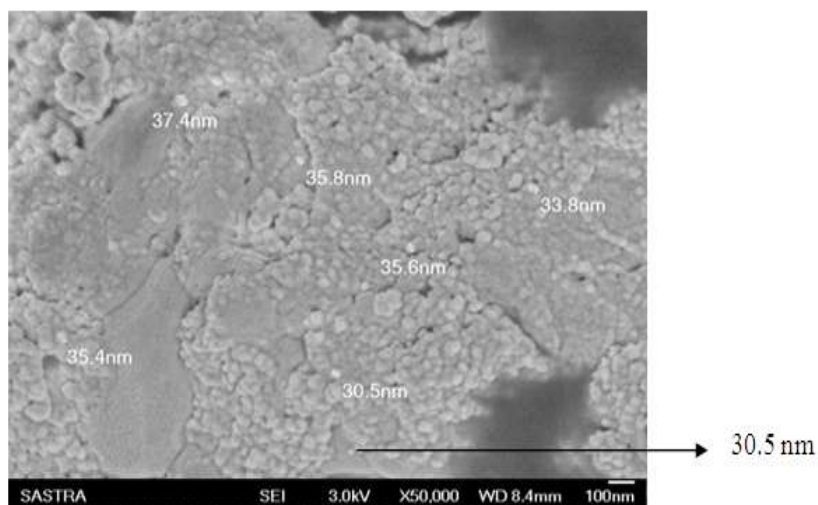


**Fig. 2:** UV-Vis absorption spectra of silver nanoparticles synthesized from *Andrographis paniculata* leaves at 3mM silver nitrate solution.

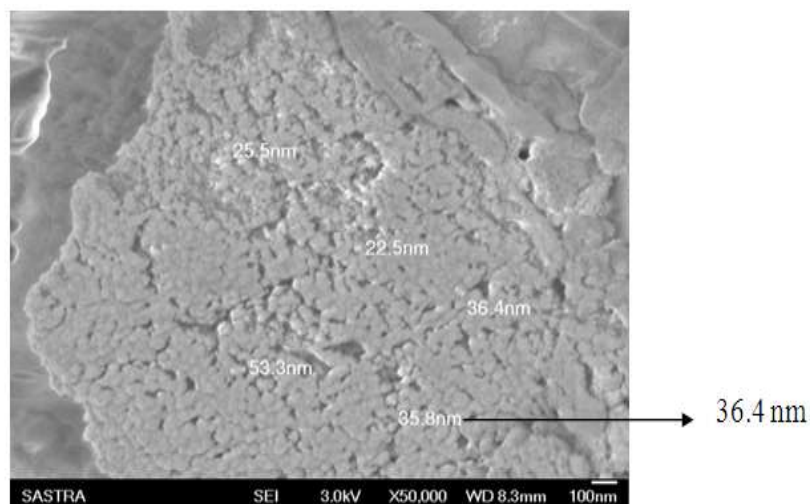
#### SCANNING ELECTRON MICROSCOPIC (SEM) ANALYSIS

The surface morphology and size of the silver nanoparticles were analysed using the SEM analysis. The SEM images shows the Silver nanoparticles synthesised from the aqueous

extract of *Andrographis paniculata* 1mM, 3mM the size ranges between 28 nm and 38 nm (Fig. 3, Fig. 4).



**Fig. 3:** SEM image of 1mM silver nanoparticles obtained from *A. paniculata*.



**Fig. 4:** SEM image of 3mM silver nanoparticles obtained from *A. paniculata*.



**Fig. 5** TEM image of *A. paniculata* 1mM

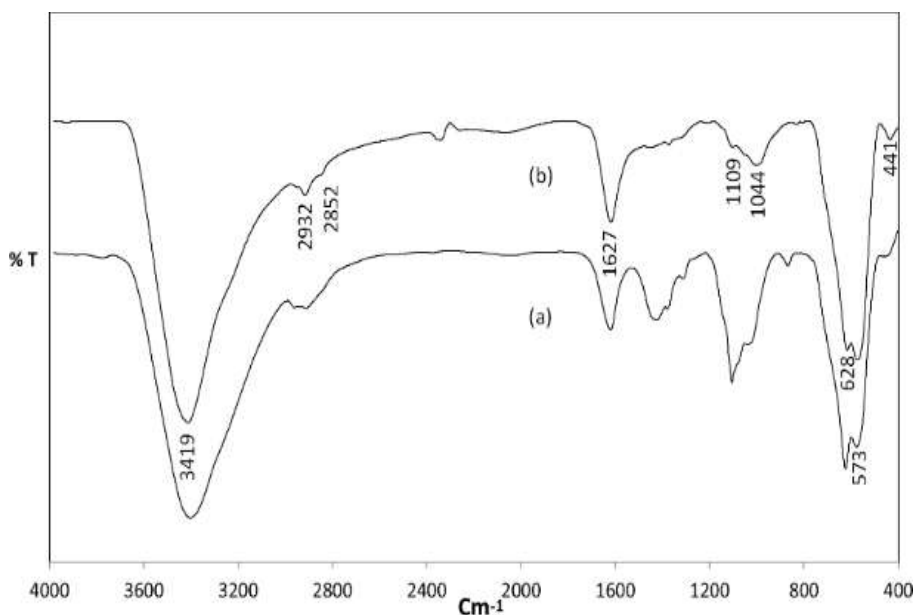


**Fig. 6 TEM image of *A. paniculata* 3mM**

### TRANSMISSION ELECTRON MICROSCOPE (TEM) ANALYSIS

Transmission electron microscopy experiment proved the formation of silver nanoparticles. The silver nanoparticles synthesised from *Andrographis paniculata* was in the range of 20 nm (Fig. 5, Fig. 6).

### FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS



**Fig.7 FTIR spectra of silver nanoparticle synthesized from *Andrographis paniculata* 1mM (a) and 3mM (b).**

Figure: 7 represents the FTIR spectrum of synthesized silver nanoparticle from *Andrographis paniculata* 1mM (a) which shows prominent absorption bands at 573, 1627, 3419  $\text{cm}^{-1}$ . Among them, the absorption peak at 1627  $\text{cm}^{-1}$  is associated with stretch vibration of C=O and is assigned to the amide I bonds of proteins. The peak at 3419  $\text{cm}^{-1}$  can be assigned as N-H stretch. This suggests the attachment of some polyphenolic components on to silver

nanoparticles. The FTIR spectrum of synthesized silver nanoparticle from *Andrographis paniculata* 3mM (b) shows prominent absorption bands at 441, 1044, 1109, 1627, 2852, 2932, 3419  $\text{cm}^{-1}$ . Among them, the absorption peak at 1627  $\text{cm}^{-1}$  is associated with stretch vibration of C=O and is assigned to the amide I bonds of proteins. The peak at 1044, 1109  $\text{cm}^{-1}$  can be assigned as C-C stretch. The peak at 3419  $\text{cm}^{-1}$  can be assigned as N-H stretch.

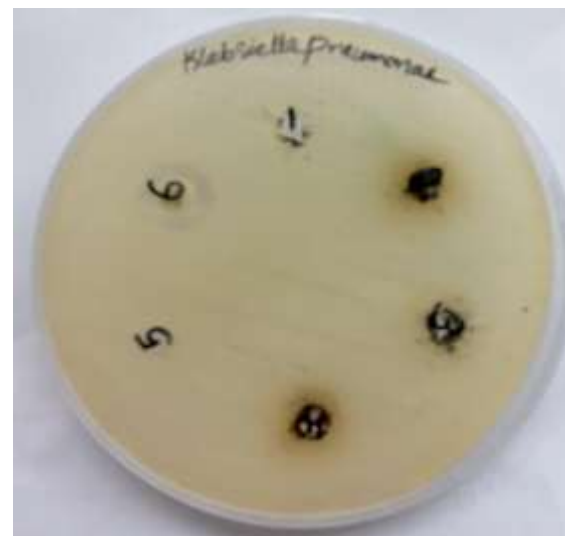
#### ANTIBACTERIAL ACTIVITY

Antibacterial activity of silver nanoparticle synthesised from, *Andrographis paniculata* and silver nitrate solution (1mM, 3mM) were observed against the microorganisms such as *Pseudomonas aurigenosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Enterococcus faecalis* in the MHA plates. The zone of inhibition obtained due to the activity of silver nanoparticle synthesized and silver nitrate solution were listed in the table 1.

Table 1. Antibacterial activity of silver nanoparticle synthesized from *Andrographis paniculata*.

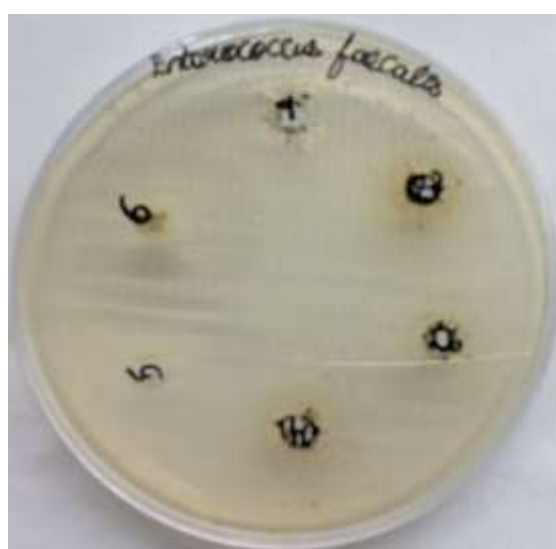
Microorganisms	Zone of inhibition							
	Silver nitrate solution		Silver nanoparticle					
	1mM	3mM	1mM <i>Andrographis paniculata</i>	3mM <i>Andrographis Paniculata</i>	1mM <i>Andrographis Paniculata</i> (duplicate)	3mM <i>Andrographis Paniculata</i> (duplicate)	Positive Control	Negative Control
<i>Pseudomonas aurigenosa</i> (Fig:8)	Nil	Nil	Nil	17mm	Nil	16mm	36mm	Nil
<i>Klebsiella pneumoniae</i> (Fig:9)	Nil	Nil	Nil	10mm	Nil	11mm	12mm	Nil
<i>Proteus vulgaris</i> (Fig: 10)	Nil	Nil	Nil	Nil	Nil	Nil	24mm	Nil
<i>Enterococcus faecalis</i> (Fig: 11)	Nil	Nil	Nil	Nil	Nil	Nil	32mm	Nil

## Antibacterial activity of synthesized silver nanoparticle

Fig.8: *Pseudomonas aurigenosa*Fig. 9: *Klebsiella pneumonia*

**Fig. 10: *Proteus vulgaris***

- 1 – *A. paniculata* 1mM
- 2 – *A. paniculata* 3mM
- 3 – *A. paniculata* (duplicate) 1mM

**Fig. 11: *Entrococcus faecalis***

- 4 – *A. paniculata* (duplicate) 3mM
- 5 – Negative control (distilled water)
- 6 – Positive Control (amoxicillin)

The figures: 8, 9, 10 and 11 represent the antibacterial activity of the silver nanoparticles, exhibited over *Pseudomonas aurigenosa*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Entrococcus faecalis*. From table: 1 the activity of silver nanoparticles synthesized from *Andrographis paniculata* 1mM showed no zone of inhibition in any species whereas 3mM showed zone of inhibition on *Pseudomonas aurigenosa* and *Klebsiella pneumoniae* in the diameter of 17mm and 10mm respectively.

The positive control (amoxicillin) showed zone of inhibition over *Pseudomonas aurigenosa*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Entrococcus faecalis* in the diameter of 36mm, 12mm 24 mm and 32mm. The negative control (distilled water) showed no zone of inhibition.

However, no zone of inhibition was found against *proteus vulgaris* and *enterococcus faecalis* except the positive control (amoxicillin).



## Antibacterial activity of silver nitrate solution

Fig. 12: *Pseudomonas aeruginosa*Fig. 13: *Klebsiella pneumoniae*Fig. 14: *Proteus vulgaris*Fig. 15: *Enterococcus faecalis*

1 – 1mM silver nitrate solution

2 – 3mM silver nitrate solution

3 – Negative Control (distilled water)

4 – Positive control (amoxicillin)

The figures: 12, 13, 14 and 15 shows the antibacterial activity of silver nitrate solution 1mM and 3mM exhibited over *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Enterococcus faecalis*. As mentioned in table: 1 there was no activity for both 1mM and 3mM silver nitrate solutions as there was no zone of inhibition found except the positive control (amoxicillin).

These results show that the silver nitrate solution of 1mM and 3mM concentration has no activity against these bacterial species.

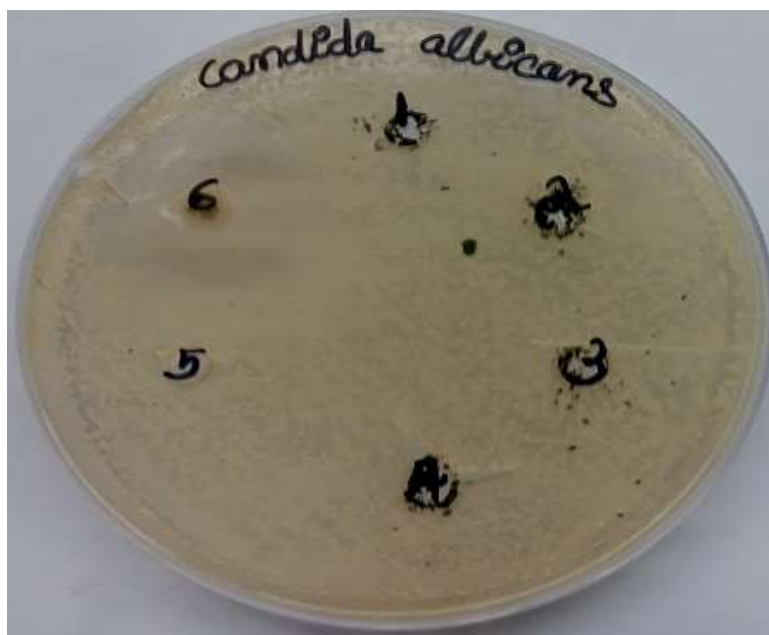
### ANTIFUNGAL ACTIVITY

The Antifungal activity of the silver nanoparticles synthesised from *A. paniculata* and silver nitrate solution (1mM, 3mM) were observed against the *Candida albicans* in the SDA plates. The zone of inhibition occurred in the *Candida albicans* were given in the table 2.

**Table. 2: Antifungal activity of silver nanoparticle synthesized from *Andrographis paniculata*.**

Microorganisms	Zone of inhibition							
	Silver nitrate solution		Silver nanoparticle				Positive Control	Negative Control
	1mM	3mM	1mM <i>Andrographis paniculata</i>	3mM <i>Andrographis paniculata</i>	1mM <i>Andrographis paniculata</i> (duplicate)	3mM <i>Andrographis Paniculata</i> (duplicate)		
<i>Candida albicans</i>	Nil	Nil	Nil	Nil	Nil	Nil	24mm	Nil

### Antifungal activity of synthesized silver nanoparticle



**Fig. 23: *Candida albicans***

- 1 – *A. paniculata* 1mM
- 2 – *A. paniculata* 3mM
- 3 – *A. paniculata* (duplicate) 1mM
- 4 – *A. paniculata* (duplicate) 3mM

5 – Negative Control (distilled water)

6 – Positive Control (amoxicillin)

The figure: 23 represent the antifungal activity of the silver nanoparticles, exhibited over *Candida albicans*. From table: 2 the silver nanoparticles synthesized from *Andrographis paniculata* 1mM and 3mM does not show any activity against the *Candida albicans* respectively. The zone of inhibition region of 24 mm occurred for the positive control (Nystatin). The zone of inhibition does not occur over the negative control (distilled water).

#### Antifungal activity of silver nitrate solution



**Fig. 24: *Candida albicans***

1 –1mM silver nitrate solution

2 –3mM silver nitrate solution

4 – Positive Control (Nystatin)

3 – Negative Control (distilled water)

The figure: 24 represent the antifungal activity of the silver nitrate solution, exhibited over *Candida albicans*. From table: 2 the silver nitrate solution of 1mM, 3mM does not show any activity against the *Candida albicans* respectively. The zone of inhibition region of 24 mm occurred for the positive control (Nystatin). The zone of inhibition does not occur for the negative control (distilled water). This results show that the silver nitrate solution does not have antifungal activity.

## SUMMARY

The synthesized silver nanoparticles from the herb *Andrographis paniculata* were identified by the colour change from green to brown colour. The colour showed the formation of silver nanoparticle and it was characterized by UV – VIS spectroscopy, SEM, TEM and FTIR analysis. The peaks obtained in the range of 435 nm for *Andrographis paniculata*.

The sizes of the silver nanoparticles under TEM analysis were ranging from 20 nm for *Andrographis paniculata*. The functional groups of the compounds absorbed on silver nanoparticles were identified using FTIR studies.

The Synthesized silver nanoparticles were checked for its antibacterial and antifungal activity. The Silver nanoparticles synthesized from *Andrographis paniculata* 3mM showed its antibacterial activity against *Pseudomonas aurigenosa* and *Klebsiella pneumonia*. It was compared with the activity of Silver nitrate solution for its antibacterial and antifungal activity, but it does not showed any activity against them.

## CONCLUSION

Synthesis of silver nanoparticle from the leaves of *Andrographis paniculata* was confirmed by the colour change from green to dark brown, which indicated the formation of the silver nanoparticles. The structural, morphological and elemental studies of biologically synthesized AgNPs were characterized by UV, SEM, TEM and FTIR respectively. The AgNPs were spherical in shape with average size ranges between 20 and 60 nm. This eco-friendly, biologically synthesized silver nanoparticles could be of immense use in medical field for their effect in antimicrobial activity. The results obtained for the nanoparticles synthesized from *Andrographis paniculata* didn't show proper antibacterial and antifungal activity. As mentioned that this project is focused on antibacterial and antifungal activity against gangrene, nanoparticles can be synthesized from various other herbs and can be used in medicine for dressing up a wound instead of maggot's therapy. The work is further preceded with nanoparticles synthesized from different plants rather than *Andrographis paniculata* and will be focused to treat gas gangrene. If positive results obtained then the silver nanoparticles will be used for treatment after undergoing interactive activity over animal cell lines.

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