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ANTIMICROBIAL ACTIVITY AGAINST GANGRENE USING SILVER NANOPARTICLES SYNTHESIZED BY *COLEUS AROMATICUS*

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ABSTRACT

Biologically synthesized nanoparticles have been widely used in the field of nanoscience and medicine. In the present investigation, nanoparticles are prepared using medicinally important plant like *Coleus aromaticus.* 1mM and 3mM silver nitrate (AgNO3) solution and the corresponding leaf extracts in ratio 1:9 were used to synthesize 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 *Coleus aromaticus* ranged at 460 nm confirms the synthesis of silver nanoparticles. SEM was used to determine the size of the nanoparticle and it was found to be in the range of 20- 60 nm. The aim of the study is to determine the antibacterial and antifungal activity of the synthesized silver nanoparticles from the leaf extracts of *Coleus aromaticus*. The most predominantly found microbial species in gangrene are *Pseudomonas auringenosa, Escherichia coli, Klebsiella pneumoniae, Clostridium perferingens, Enterococcus faecalis, Candida albicans, Staphyllo coccus.* Agar well diffusion method was used to check the activity of silver nanoparticle against gangrene causing microbes. 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: *Coleus aromaticus, Clostridium perferingens, Candida albican,* silver nanoparticle, SEM, TEM, FTIR.

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.

Novel applications of nanoparticles and nanomaterials 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. Such two plants were selected for study as nanodrug. The plant used for the treating the gangrene infection is *Coleus aromaticus.*

Coleus aromatics (Lamiaceae) is commonly known by different folk names such as Country-borage, Indian-borage (English), Ajwain patta, Ommavalli (Tamil), Pattharachuur (Bengal), Bangun-bangun leaves, Indian-mint, French-thyme, Mexican-mint, Soup-mint, Spanish-

thyme, Oreille, Hung chanh, Maxianthyme, which confirms its large diffusion in tropical areas. In Ayurvedic medicine, the plant is known as Parna- Yavaani.

Coleus aromaticus looks as a green, perennial, shrub having heart shaped and leathery leaves with scalloped edges. The plant grows to around 50 cm tall with horizontal stems up to 180 cm long. The leaves are highly aromatic with a strong flavour of mixed herbs and make an excellent addition in stuffing for meat and poultry. Finely chopped, they can also be used to flavour meat dishes, especially beef and lamb. *Coleus aromaticus*, a plant of medical and food interest is told as "must- have" plant in medicinal herb garden. It is known to possess antimicrobial, antiepileptic, leishmanial, and antioxidant activities. The leaves are also used for treatment of cough, throat infection and nasal congestion. Daily chewing of a leaf is recommended for all age groups; especially during winter to ward off cold.

MATERIALS AND METHODS

PREPARATION OF SAMPLE

Collection of Samples

The fresh leaves of *Coleus aromaticus* (Omavalli) were collected from the region of Tambaram, Chennai.

Aqueous extract Preparation

Materials

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

Methodology

The fresh leaves of *Coleus aromaticus* was washed with distilled water and were dried for few minutes in room temperature. 100g of fresh leaves of *Coleus aromaticus* were weighed, grinded completely using mortar and pestle and aqueous extract was filtered using whatman filter paper No. 1. The aqueous extract of *Coleus aromaticus* were used for the further synthesis process.

Boiled extract Preparation

Materials

Fresh leaves of *Coleus aromaticus* and, distilled water, beaker, microwave oven (LG), whatman filter paper No. 1.

Methodology

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

Coleus aromaticus Boiled extracts, 1mM silver nitrate solution, 3mM silver nitrate solution, conical flask, and beaker.

Methodology

To 5ml boiled extract of *Coleus aromaticus* 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 formed in the boiled extract of *Coleus aromaticus.* Hence the further work was preceded with the synthesis of silver nanoparticles obtained from the aqueous extract of the *Coleus aromaticus.*

SYNTHEIS OF SILVER NANOPARTICLES

Synthesis of 1mM silver nanoparticles

Materials

Coleus aromaticus aqueous extract, 1mM silver nitrate solution, beaker, conical flask, measuring cylinder, centrifuge tubes, test tubes, centrifuge.

Methodology

To 5ml aqueous extract of *Coleus aromaticus* 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 *Coleus aromaticus* 3mM silver nitrate solution, beaker, conical flask, measuring cylinder, centrifuge tubes, test tubes and centrifuge.

Methodology

To 5ml aqueous extract of *Coleus aromaticus* 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 Petri plates 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 *Coleus aromaticus* 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 *Coleus aromaticus* 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 cm−1 at a resolution of 4 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, Entrococcus faecalis,* Muller Hinton Agar (MHA), peptone water, test tubes, petriplates, inoculation loop, laminar air flow chamber, distilled water, samples (1mM, 3mM *Coleus aromaticus*), antibiotic disc, bunsen burner, micropipette, cotton, micropipette tips, gel

puncture, incubator (Yorco), Autoclave (Yorco), conical flask, Dhona electronic balance and standard (Mc Farland).

Methodology

Subculturing was done from the stock culture of *Pseudomonas aurigenosa, Klebsiella pneumoniae, Proteus vulgaris, Entrococcus 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 1µg (1mM, 3mM *Coleus aromaticus*) 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, Entrococcus faecalis,* Muller Hinton Agar (MHA), peptone water, test tubes, petriplates, inoculation loop, laminar air flow chamber, distilled water, 1mM and 3mM $AgNo_3$ 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, Klebsilla pneumonia, Proteus vulgaris, Entrococcus 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 $AgNo_3$ 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 *Coleus aromaticus* and *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 *Coleus aromaticus* and *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 $AgNo_3$ 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 $AgNo_3$ 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 around 400 nm in case of *Coleus aromaticus* 1mM and 3mM (Fig. 1) (Fig. 2), From different literatures studies it was observed that the silver nanoparticles shows peak at around 460 nm for *Coleus aromaticus.* The peak formation in the range of 400 nm to 440 nm clearly indicates the synthesis of silver nanoparticles from the aqueous leaves extract.

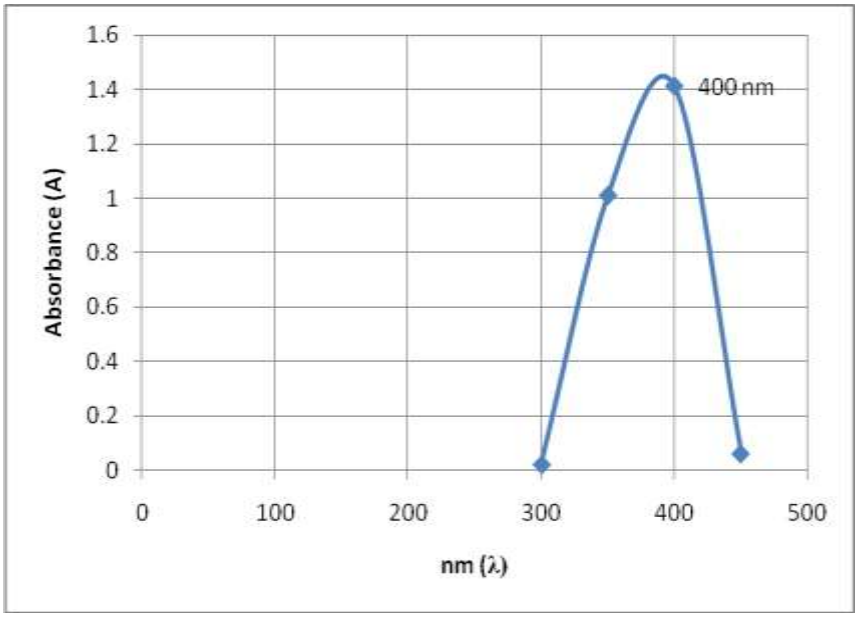

Fig. 1: UV-Vis absorption spectra of silver nanoparticles synthesized from *Coleus aromaticus* leaves at 1mM silver nitrate solution.

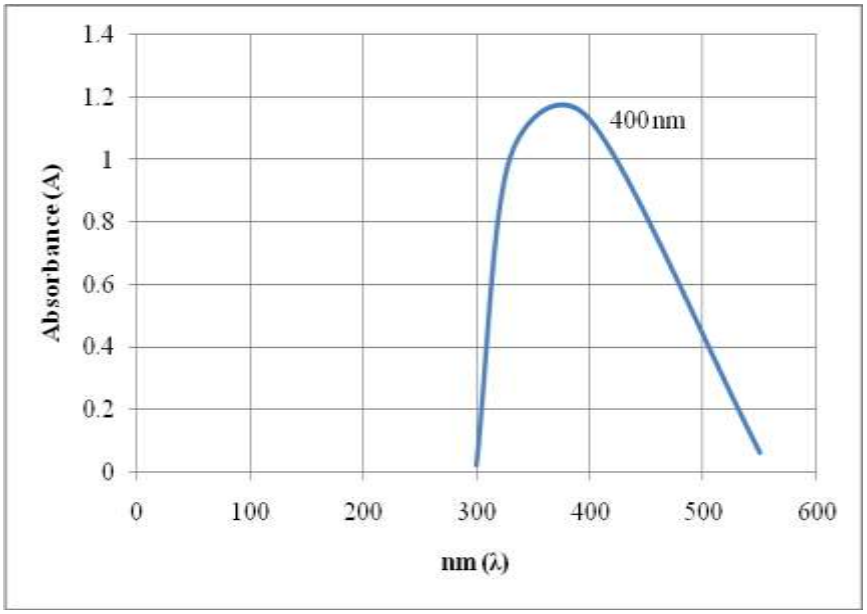

Fig. 2: UV-Vis absorption spectra of silver nanoparticles synthesized from *Coleus aromaticus* leaves at 3mM silver nitrate solution.

SCANNING ELECTRON MICROSCOPIC (SEM) ANALYSIS

The surface morphology and size of the silver nanoparticles were analysed using the SEM analysis. The SEM images shows a size range of 23nm-38nm for the Silver nanoparticles synthesised from the aqueous extract of *Coleus aromaticus* 1mM, 3mM (Fig.5, Fig. 6).

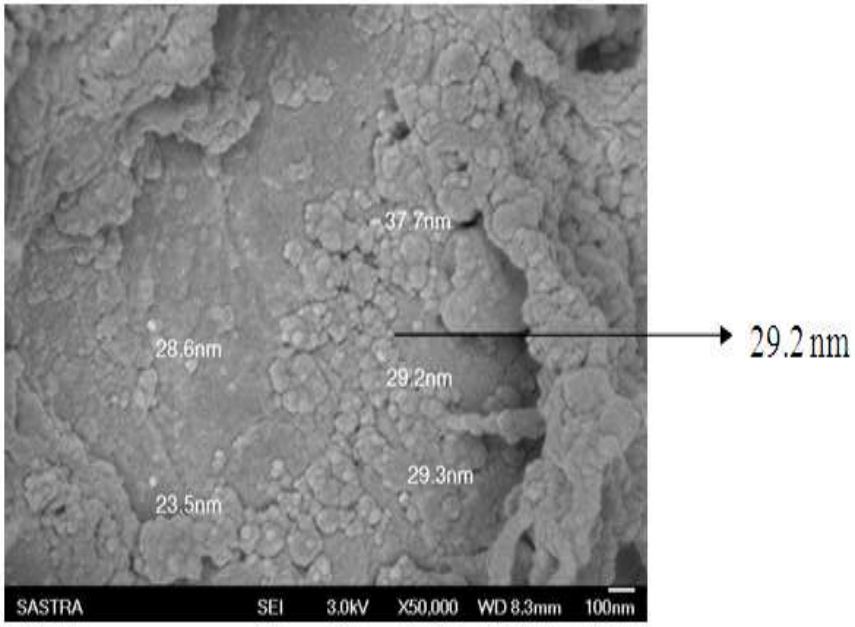

Fig. 5: SEM image of 1mM silver nanoparticles obtained from *C. aromaticus.*

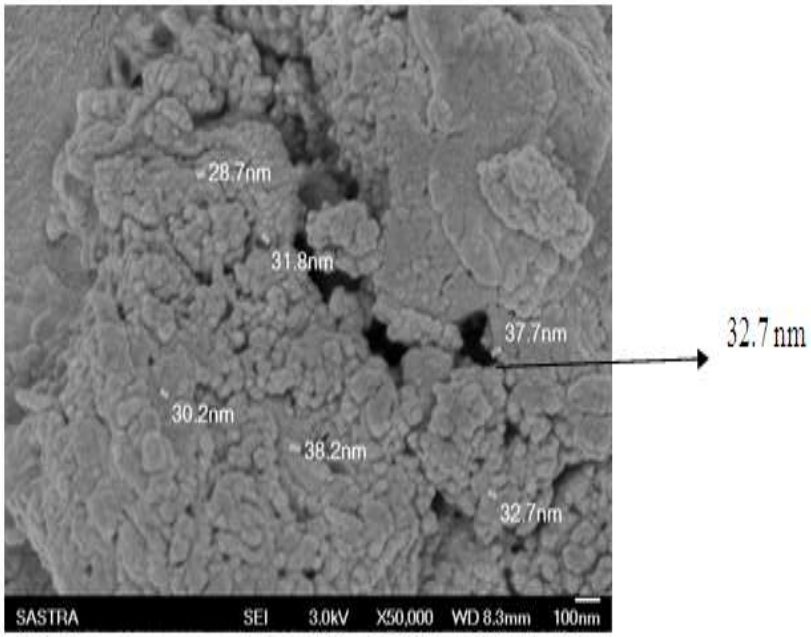

Fig. 6: SEM image of 3mM silver nanoparticles obtained from *C. aromaticus.*

TRANSMISSION ELECTRON MICROSCOPE (TEM) ANALYSIS

Transmission electron microscopy experiment proved the formation of silver nanoparticles. The silver nanoparticles synthesised from *Coleus aromaticus* was in the range of 25 nm (Fig. 9, Fig. 10).

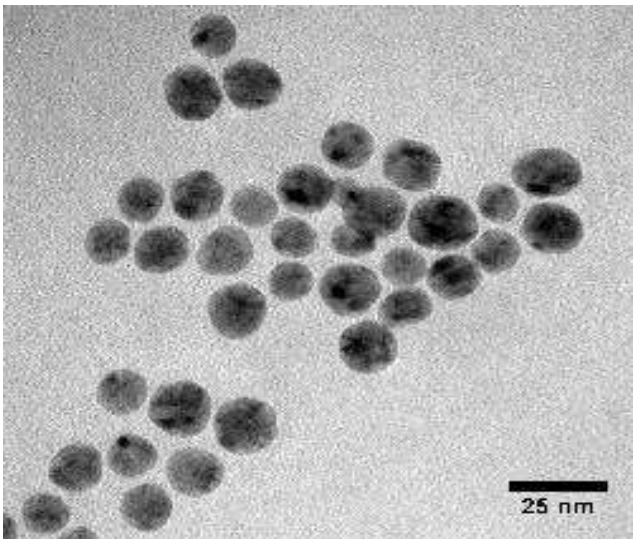

Fig. 9 TEM image of *C. aromaticus* 1mM

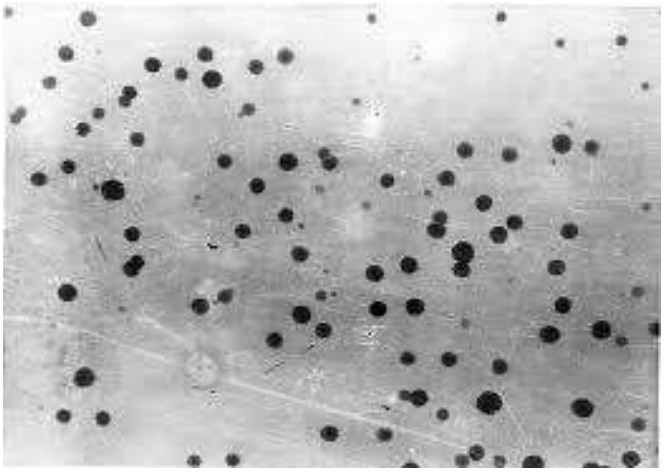

Fig. 10 TEM image of *C. aromaticus* 3mM

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS

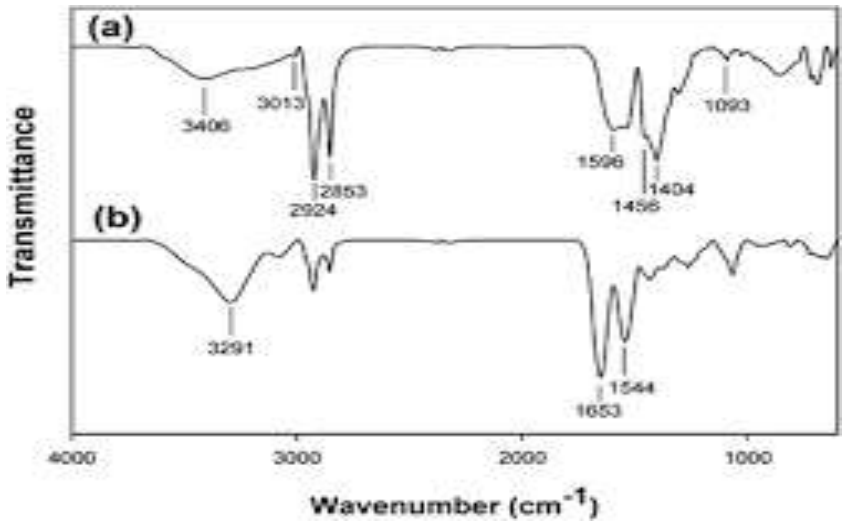

Fig. 13 FTIR spectra of silver nanoparticle synthesized from *Coleus aromaticus* 1mM (a) and 3mM (b)

Figure: 13 represents the FTIR spectrum of synthesized silver nanoparticle from *Coleus aromaticus* 1mM (a) which shows prominent absorption bands at 1093, 1404, 1456, 1506, 2853, 2924, 3015, 3406 cm^{-1}. Among them, the absorption peak at 2924cm^{-1} can be assigned as absorption peaks of CH stretch several bands (alkyl), the peak at 1506 cm^{-1} is associated with stretch vibration of -C = C- and is assigned to the amide 1 bonds of proteins. The peak at 1093 can be assigned as C-O stretch (CH-O-H in cyclic alcohols). The peak at 2853, 2924, 3015, 3406 can be assigned as N–H stretch. This suggests the attachment of some polyphenolic components on to silver nanoparticles.

Figure: 13 also represents the FTIR spectrum of synthesized silver nanoparticle from *Coleus aromaticus* 3mM (b) which shows prominent absorption bands at 1544, 1653, 3291 cm^{-1}.

Among them, the absorption peak at 1544, 1653 cm^{-1} is associated with stretch vibration of -C = C- and is assigned to the amide 1 bonds of proteins. The peak at 3291 can be assigned as C–H stretch. This suggests the attachment of some polyphenolic components on to silver nanoparticles.

ANTIBACTERIAL ACTIVITY

Antibacterial activity of silver nanoparticle synthesised from C*oleus aromaticus and* silver nitrate solution (1mM, 3mM) were observed against the microorganisms such as *Pseudomonas aurigenosa, Klebsiella pneumoniae, Proteus vulgaris, Entrococcus 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 *Coleus aromaticus* and *Andrographis paniculata.*

Microorganisms	Zone of inhibition							
	Silver nitrate solution		Silver nanoparticle					
	1mM	3mM	1mM *Coleus aromaticus*	3mM *Coleus aromaticus*	1mM *Coleus aromaticus* (duplicate)	3mM *Coleus aromaticus* (duplicate)	Positive Control	Negative Control
Pseudomonas aurigenosa (Fig:9)	Nil	Nil	16 mm	15mm	15.5mm	15.5mm	36mm	Nil
Klebsiella pneumoniae (Fig:10)	Nil	Nil	10mm	11mm	10.5mm	11mm	12mm	Nil
Proteus vulgaris (Fig: 11)	Nil	Nil	Nil	Nil	Nil	Nil	24mm	Nil
Entrococcus faecalis (Fig: 12)	Nil	Nil	Nil	Nil	Nil	Nil	32mm	Nil

Antibacterial activity of synthesized silver nanoparticle

Fig. 15: *Pseudomonas aurigenosa*

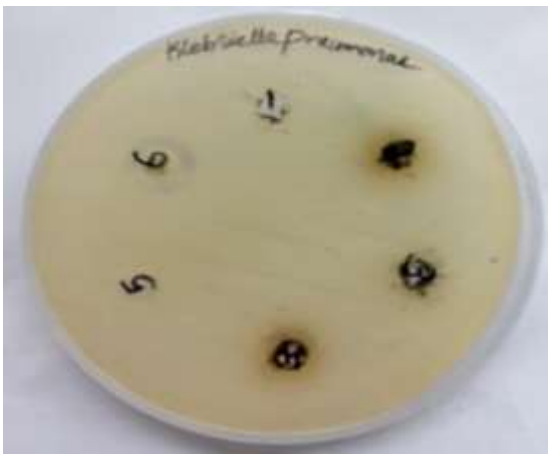

Fig. 16: *Klebsiella pneumoniae*

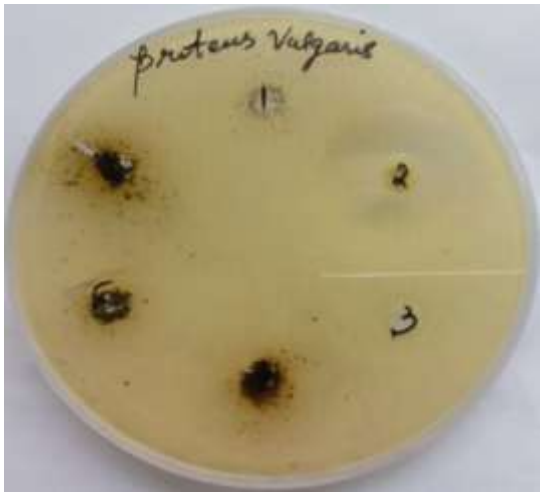

Fig. 17: *Proteus vulgaris*

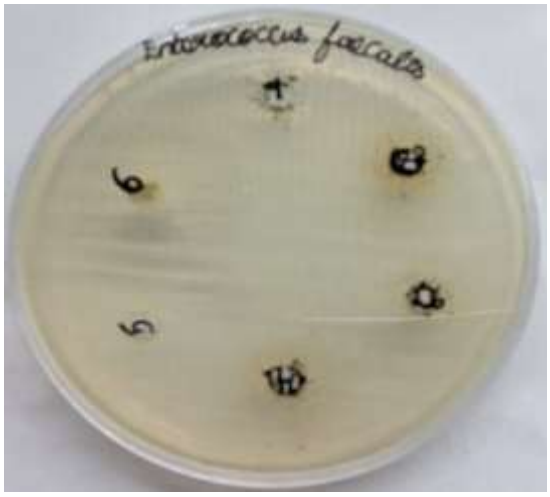

Fig. 18: *Entrococcus faecalis*

1 – *C. aromaticus* 1mM

2 – *C. aromaticus* 3mM

3 – *C. aromaticus* 1mM(duplicate)

4 – *C. aromaticus* 3mM(duplicate)

5 – Negative control (distilled water)

6– Positive Control (amoxicillin)

The figures: 15, 16, 17 and 18 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 *coleus aromaticus* 1mM showed the zone of inhibition in the diameter of 16mm and 10mm, over *Pseudomonas aurigenosa* and *Klebsiella pneumoniae* and for 3mM it showed the zone of inhibition in the diameter of 15mm and 11mm 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 19: *Pseudomonas aurigenosa*

Fig. 20: *Klebsiella pneumonia*

Fig. 22: *Proteus vulgaris*

Fig. 21: *Entrococcus faecalis*

1 – 1mM silver nitrate solution

3 – Negative Control (distilled water)

2 – 3mM silver nitrate solution

4 – Positive control (amoxicillin)

The figures: 19, 20, 21 and 22 shows the antibacterial activity of silver nitrate solution 1mM and 3mM exhibited over *Pseudomonas aurigenosa, Klebsiella.* 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 *C. aromaticus*, *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 *Coleus aromaticus.*

Microorganisms	**Zone of inhibition**							
	Silver nitrate solution		**Silver nanoparticle**					
	1mM	**3mM**	**1mM** *Coleus aromaticus*	**3mM** *Coleus Aromaticus*	**1mM** *Coleus Aromaticus* (duplicate)	**3mM** *Coleus Aromaticus* (duplicate)	**Positive Control**	**Negative Control**
Candida albicans	Nil	Nil	Nil	Nil	Nil	Nil	24mm	Nil

Antifungal activity of synthesized silver nanoparticle

Fig. 23: *Candida albicans*

1 – *C. aromaticus* 1mM

2 – *C. aromaticus* 3mM

3 – *C. aromaticus* 1mM(duplicate)

4 – *C. aromaticus* 3mM(duplicate)

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 *coleus aromaticus* 1mM and 3mMm does not show any activity against the *Candida albicans* respectively. The zone of inhibition region of 24 mm occurred for the positive control (Nystanin). 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 –1mM silver nitrate solution

3 – Negative Control (distilled water)

4 – Positive Control (Nystatin)

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 (Nystanin). 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 *Coleus aromaticus* was 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 400 nm for *Coleus aromaticus.*

The sizes of the silver nanoparticles under TEM analysis were ranging from 25 nm for *Coleus aromaticus.* The functional groups of the compounds absorbed on silver nanoparticles were identified using FTIR studies.

The result obtained is that the silver nanoparticles synthesized from *Coleus aromaticus* showed antibacterial activity against *Klebsiella pneumoniae* and *Pseudomonas auringenosa.* These results were then compared with antibacterial and antifungal activity of silver nitrate solution, which didn't exhibit any activity against bacteria or fungi.

CONCLUSION

Synthesis of silver nanoparticle from the leaves of *Coleus aromaticus* 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 *Coleus aromaticus* 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 *Coleus aromaticus* 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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