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PHYTOCRYSTALLIZATION OF SILVER NANOPARTICLES USING CASSIA ALATA FOR EFFECTIVE CONTROL OF FUNGAL SKIN PATHOGENS

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Abstract:
The primary objective of the present study is to synthesis and characterization silver nanoparticles using *Cassia alata* aqueous flower extract and investigate the antifungal activity and cytotoxicity study. *Cassia alata* aqueous flower extract have been effectively used for the synthesis of silver nanoparticles and demonstrated the efficacy of *Cassia alata* flower as natural renewable and low costing bioreduction agent of silver. The characterizations of AgNPs such as UV-visible, FTIR, SEM, EDX and XRD. We have synthesized AgNPs of 20 nm in size with spherical shape using aqueous extract of *Cassia alata.* AgNPs synthesized by flower extract were very distinct with very small size, crystalline nature and clearly proved their biomedical importance by exhibiting strong antifungal activity against skin pathogens. In addition cell line study reveals that low concentration of AgNPs was showed a 98% cell viability. Thus AgNPs possess important applications in biomedical or pharmaceutical industry for the preparation of antifungal skin ointments.

Key words: Silver nanoparticles, Antifungal, *Cassia alata*, Human Fibroblast cell line

1. INTRODUCTION

Biomedical remediation of skin disease using nano synthesized silver particle have easily accessible frequent fresh and economical yield growing technology applications. At the special properties have been presenting in the synthesized nanoparticles and have superior bio activity then larger particles resulting in greater utilization single cell tissue and organ. The nanoparticles (NPs) run in to the body penetrate and cause injury to biological membrane, cell and nuclei [12]. Based on the advanced nanomaterials of noble metals like silver has conquered a lot of interest among scientists during the past periods for its physiochemical properties such as size, distribution and morphology, they have been studied for catalytic activity electronic properties, antibacterial properties and magnetic properties [22].The delivery of drug through the skin as long been promising concept because of the ease of access, large surface area, vast exposure to the circulatory and lymphatic networks and non-invasive nature of the treatment [8].

The development in challenge of cataly NPs to make a nanomaterial's are high active and inexpensive [24]. The replace the chemical synthetic procedures with clean nontoxic. The acceptance of environmentally biological synthesis. Many researchers have been towards the biological organization as microorganism and plant to magnet motivation for biological synthesis. To the metal

base NPs that stand synthesized for several application. Since the extracts of deferent part of the plant such as. Root, stem, leaves, flower, fruit, seed, etc., some of the plants have more and more presenting the water content and co-enzyme as presented in the plant extract that uses of reduce metal ions from the NPs in a single step for green synthesis nanoparticles [16]. Then the synthesized AgNPs reproducible and very constant of metal, metal oxides and the complex of NPs chemical decrees the same time using biosynthesizes material as a reducing is relatively rapid, viable at room temperature and pressure, nontoxic simply mounted in environment hereafter this follow by the green chemistry procedure as involved the action of biologists and nanotechnologists and take recently developed as one of the now present of the areas nanobiotechnological [10].Preparation of extract varies part of the plant and have used well synthesize nanoparticle.

The aqueous leaf extract various pharmacological properties such as anti-bacteria, anti-fungal as well as anti-inflammatory activity [20]. Leaf sap contains a fungicide, chrysophanic acid used to treat several skin ailments [3] from the leaves of *Cassia alata (C. alata)* are used as an effective treatment against ringworm and also against other skin ailments such as eczema and chronic skin impurities [14]. *C.alata* leaves containing more phytochemical (emodin, kaempferol, aloe-emodin, chrysophanol and iso-chrysophanol, rhein, ellagitannin phenolic acid and cassia xanthone among the other substances. Astragalin is important phytochemical presenting in *C. alata* leaves [19]. The recent studies exposed that *C. alata* has been proven to be affective agent bacteria and fungi [8] observed that the minimal inhibitory concentration values of synthesized silver nanoparticle of leaves of *C.alata* against the bacteria and fungi [40]. Presence of preliminary phytochemical analysis of *C.alata* showed the presence of phenol, tannins, anthraquinones, saponins and flovonods.[4]. the environment technology including energy conversion, chemical development and biological usage. To the plant material present type of nanoparticles is classified in inorganic metals in (silver, gold and etc.,) metal oxides of (ZnO2, CeO2, TiO2, iron oxides and etc.,) carbon based nanoparticles (e.g grapheme, fullerenes and carbon nanotube). some physical properties of metal NPs have been special application by the control based on the size, shape and structure [7].

The synthesized nanoparticle have been presented in stable metal with controlled size and shape, there has been search for economical, safe, and reliable and "green" approach. The novel methods so called green biosynthesis have been recently developed by a variety of plant extract [18]. Presence of some toxic compound absorbed on the surface of the nanoparticals. These are synthesized by chemical methods may have adverse effect on the biomedical applications. Therefore, 'green synthesis' method, than the chemical and physical method. Green synthesis is effective, eco-friendly and without trouble scaled to the large scale. Not available of high pressure, energy, temperature and toxic chemicals. The inhibitory effects of silver on microbes, which was identified in the older, the nanopartical widely usually used in medical and industrial processes [28]. The common name of the *Cassaia lata* is candle bush, wild senna, ringworm senna, and etc.., it belongs to the family Caesalpiniaceae, a tropical annual herb consisting of leathery, and compound leaves. The leaves have laxative properties and very good antimicrobial and antifungal activity. The leaves are extensively used against dermatophyte infections, such as 'tide a foot, popularly known as 'athlete foot, for superficial mycoses and also in the treatment of chronic fungal infections such as 'pityriasisversicolor'. They are used externally for the treatment of skin disease, acne, tinea infections, insect bites, ringworms, eczema, scabies, itchiness, and internally as expectorant for bronchitis, for alleviation of asthma symptoms, as a laxative to expel intestinal parasites, for stomach problems and weight loss[6]

Cassai alata was commonly used as medicinal plant of India and Southeast Asia this plant physical properties of anti-inflammatory, hydragogue, sudorific, diuretic, pesticide, Root, leaves, flower, and seed of this plant own biological property such as antibacterial, antifungal, antitumor and urinary tract infections [36]. Asthma bronchitis and constipation [31].*C.alalta* used against to yellow fever or malaria, and its antiasthmatics of antidiabetics[17]. Fresh leaf gently grain and apply to the ringworm, skin diseases, itching, mycosis, atlati food disease, impetigo, syphilis sores and eczema. *C. alata*

mainly presented are flavonoids, alkaloids, tannins, anthraquinone derivatives, sterols and triterpenes[39]. Reports of evidence of the literature that plant synthesized silver nanopartical (AgNPs) using the plant family such as C*assia amgustifo Cassia auriculata, Cassia fistula, Cassia italic and Cassia torai. C.alata* extract leaves reducing the free radical component of antioxidant property [1]. The present work is aimed to synthesize silver nanoparticle using aqueous flower extract of *C. alata* and characterize the synthesized AgNPs and evaluate *in vitro* effect of AgNPs on fungal skin pathogen.

2. MATERIALS AND METHODS

2.1. Collection of plant sample:

The *Cassia alata* plant flower were freshly collected from Enathur village (latitude 12.84 N and the longitude is 79.73 E) Kanchipuram at Tamilnadu in India.

2.2. Preparation of plant aqueous flower extract:

The *cassia alata* flower were cut into small pieces in used sterile scissors and washed surface of flower using sterile distilled water. The removed surface dusts. About 50g of flower were soaked 100 ml distilled water it's boiled in heating mantle for 30 min at 100ºC using 500 ml beaker. After the boiling extract was cooled, filtered with muslin cloth and then Whatman No. 1 filter paper used. This extract freshly prepared each experiment. Extract was stored at 4°C for further uses.

2.3. Phytochemical analysis of *Cassia alata* flower extract:

The rich in important phytochemical constituents such as flavonoids, phenols, terpenoids, glycosides, anthraquinones and quinones analysis of phytochemical [26].

2.3.1. Test for flavonoids:

To 1ml of extract, 2ml of distilled water and few drops of 10% aqueous $Fecl_3$ were added. Formation of brown colour indicates the presence of flavonoids.

2.3.2. Test for phenols:

To 1ml of extract, 2ml of distilled water and few drops of 10% aqueous $Fecl_3$ were added. Formation of blue or green colour indicates the presence of phenols.

2.3.3. Test for terpenoids:

5ml of extract were mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added along the sides of the test tube. A reddish brown colour at the interface indicates the presence of terpenoids.

2.3.4. Test for glycosides:

1ml of the extract was dissolved in 1ml of glacial acetic acid and cooled then add 2-3 drops of ferric chloride. To this 2ml of concentrated H_2SO_4 was added carefully along the walls of the test tube. Appearance of reddish brown ring at the junction of two layers indicates the presence of glycosides.

2.3.5. Test for anthraquinones:

To 1ml of extract, 2N Hydrochloric acid was added and the mixture was heated in water bath for 15 minutes, cooled and filtered. The filtrate was mixed with layer of chloroform and was separated with it. These mixtures were treated with 10% KOH solution, the aqueous layer becomes pink-red, which confirms the presence of anthraquinones.

2.3.6. Test for quinones:

To 1ml of extract 1ml of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinones.

2.4. Biosynthesis of silver nanoparticles:

Silver nitrate solution of 0.1mM were used for the bio-synthesis of silver nanoparticles. 1ml of aqueous flower extract was added to 9 ml of 0.1mM silver nitrate solution and mixed well under room temperature. A brown colour change was observed. The bio-reduction of silver ions in the solution was monitored periodically by measuring in UV-Visible spectroscopy (300 nm to 800 nm). The formation of a reddish brown coloured solution indicated the formation of the silver nanoparticles [38].

2.5. Optimization of green synthesis of silver nanoparticles:

2.5.1. Effect of different pH:

To 1.0 ml of *C. alata* flower aqueous extract and 9.0ml of 0.1mM silver nitrate was added and maintained at different pH (4, 5,6,7,8, 9and10)at room temperature and observed for brown colour changes. The absorbance of the resulting solutions was measured between 300 and 800 nm using UV-Vis spectrophotometer [25].

2.5.2. Effect of different concentration of silver nitrate:

To 1.0ml of *C.alata* flower aqueous extract and 9.0ml of 0.1mM silver nitrate was added and maintained at different pH (4,5,6,7,8, 9and10) at room temperature and observed for brown colour changes. The absorbance of the resulting solutions was measured between 300 and 800nm using UV-Vis spectrophotometer [25]

2.5.3. Effect of different concentration of substrate (flower extract):

To 1.0ml of *C. alata* flower aqueous extract 9.0ml of silver nitrate (pH9.0) were added at different concentrations such as 100mM, 200mM, 300mM, 400mM, and 500mM and incubated at room temperature. The absorbance of the resulting solutions was measured UV-Viss-pectrophotometer. [25].

2.5.4. Mass production of silver nanoparticles:

The mass production of silver nanoparticles was synthesised in optimized pH, substrate and silver nitrate concentration were used. After the mass production silver nanoparticles obtained from the solution were purified by repeated centrifugation at 12,000 rpm for 30 minutes. The pellet was dried in hot air oven and were used to characterization analysis.

2.6. Characterization of synthesized silver nanoparticles:

2.6.1. UV-Vis spectra analysis:

The synthesized bio-reduced sample was characterized by using UV-visible spectrophotometer based on various concentration of pH, substrate and silver nitrate analyzed. The sample were taken in a quartz cuvette and optical density observed at the 300-800 nm wavelengths and results were observed. [25].

2.6.2. Fourier Transform Infra-Red (FTIR) Spectra analysis:

The Fourier Transforms Infrared (FTIR) spectroscopy was used to identify the biomolecules present in the synthesized silver nanoparticles using flower extract of *C. alata*. The FTIR was recorded in range of 500 to 4000 cm^{-1}. The various modes of vibrations were identified and assigned to determine the identified functional group presents in the AgNPs[37].

2.6.3. Transmission Electron Microscope (TEM) analysis:

Size, shape and particle size distributions were determined using a JEOL JEM-2011 transmission electron microscope operated at an accelerating voltage of 200kV. Images were recorded using a Gatan Dual Vision 600t CCD camera attached to the microscope and were analyzed using Gatan Digital Micrograph Version 3.11.1. The TEM was calibrated for diffraction and imaging mode using standard samples. Their solution of the system was calibrated with manganese (Mn). Samples were

prepared for TEM analysis by placing a drop of the solution on a carbon coated copper grid and drying in air [2].

2.6.4. Energy dispersive analysis X-ray (EDX):

Energy dispersive analysis X-ray (EDX) spectrometer takes advantage of the photon nature of the light. In the X-ray range the energy of a single photon is just sufficient to produce a measurable pulse X-ray. A semiconductor material is used to detect the X-ray along with processing electronics to analysis the spectrum [13].

2.6.5. X-ray diffraction (XRD) analysis:

The crystalline nature of the green synthesized AgNPs by *Cassia alata*flower extract was also confirmed by x-ray diffraction patterns (XRD) analysis. The size, phase identification and crystalline nature of the AgNPs were determined by the XRD analysis. The characterization of the synthesized AgNPs was conducted with an X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with CuKα1 radiation in θ-2θ configurations. The crystallite domain size was calculated from the width of the XRD peaks by assuming that they were free from non-uniform strains and using the Scherrer's formula,$D = 0.94\ \lambda / \beta \cos \theta$

Where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) and θ is the diffraction angle [15].

2.7. Antifungal activity of flower extract and synthesized silver nanoparticles:

2.7.1. Test fungal strains:

The pathogenic fungi *Trichophytonrubrum, Aspergillus fumigates, Candida albicans, Candida immitis* and *Xerophilicdermatitidis)* were obtained from Rajah Muthiah Medical College and Hospital (RMMCH), Annamalai nagar and maintained on Sabouraud's dextrose agar.

2.7.2. Well Diffusion Method:

The Sabuoraud dextrose agar (SDA) plates were preparedaccording to the manufacturer's specification and allowed to cool for culturing the microorganisms. Holes were bored on the culture media plate usinga sterile well puncher5 mm in diameter which was labelled cured extract and synthesized nanoparticle. Two well were filled with crude extract (25µl and 50µl) other two well with AgNPs (25µl and 50µl).5µl of nystatin were used as the positive controls respectively with triplicates. The plates were allowed for an hour to diffuse and then incubated for 24 hours (*Candida albicans*) and 120 hours (*Trichophytonrubrum, Aspergillus fumigates, Candida immitis*and*Xerophilicdermatitidis*) after which they were examined for zones of inhibition and readings were taken in millimeters.[33].

2.7.3. Determination of Minimum Fungicidal concentration

To determine the MFCs, 1 ml of standardized inoculums spores of 1×10^7cfu/ml was mixed with Saboroud Dextrose broth and subsequently adds the different concentrations of flower extract from 10-100 µg/ml. Then all the broths were incubated in aseptic conditions at 37ºC for 24 to 48 hrs. After 48 hrs 0.1 ml of inoculums was withdrawn from each concentration contained broth and inoculated on Sabouraud dextrose agar for the examination of MFC. Plates were incubated at 37ºC for 72 h. MFC was defined as the lowest drug concentration that showed lessthan 3 colonies or no visible growth on the plates is considered as a inhibition activity of 99% or 100%, respectively [35].

2.7.4. MTT assay using Human Fibroblast cell line L929

The MTT assay is a standard colorimetric non-radioactive assay for measuring viable cell and cytotoxicity through increased metabolism of the tetrazolium salt. Skin cells (1 x 106 cells/mL) were seeded into 96 well plates and incubated for 24 hours incubation. Then the cells were treated with different concentration of drug formulation (10-200 ug/ml). Then, the cells were incubated in the presence of 5% Co_2 at 37 °C for 24 h. After incubation, MTT (0.5 mg/mL) was added to the incubated

cells. Then cells were incubated for another 4 h. Then 100 µL of DMSO were added into each well and mixed well. Absorbance was measured in a multimode reader at 570 nm.

3. RESULTS AND DISCUSSION

3.1. Collection of plant sample:

The *C. alata* plant flowerwere freshly collected from Enathur village (latitude 12.84 N and the longitude is 79.73 E) Kanchipuram at Tamilnadu in India **(Fig.1)**

Fig.1. Collection of *Cassia alata* flower:

5.1.1. Botanical classification of *Cassia alata*:

*Cassia alata*also known as *Sennaalata* **(Fig.2).**

3.2. Phytochemical analysis of *Cassia alata* flower extract:

Kingdom	*Plantae*
Order	*Fabales*
Family	*Fabaceae*
Subfamily	*Caesalpinioideae*
Tribe	*Cassieae*
Subtribe	*Casslinae*
Genus	*Senna*
Species	*Alata*
Botanical name	*Sennaalata/Cassia alata*

The presence of Flavonoids, Phenols, Cardiac glycosides, Terpenoids, Glycosides, Quinones (Table.1)were observed in aqueous flower extraction of *Cassia alata.* It has been reported that *cassia alata* is rich in many phytochemical constituents that are responsible for the immune stimulating property. Similar result was reported in the same plant *Cassia alata* including anthraquinones which was absence in present study [26].

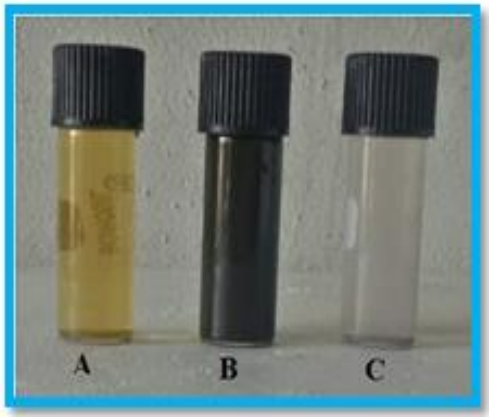
Table.1. Phytochemical analysis of *Cassia alata* flower extract:

S.No	Test	Results
1	Flavonoids	+
2	Phenols	+
3	Cardiac glycosides	+
4	Terpenoids	+
5	Glycosides	+
6	Anthraquinones	-
7	Quinones	+

3.3. Biosynthesis of silver nanoparticles:

The reduction of silver nitrate into AgNPs during exposure to plant extracts is followed by a gradual increase in colour development from clear reddish brown (Fig.3) as a results surface plasmonresonance phenomenon. The flower extracts without $AgNO_3$ did not show any color change

Fig.3. Screening of biosynthesis of silver nanoparticles:



A) Aqueous flower extraction B) Synthesized AgNPs and C) Silver nitrate solution

3.3.1. Effect of different pH:

The brown colour changes was observed (Fig.4a) in various pH from 2-10. The absorbance of the resulting solutions was measured between 300 and 800n musing UV-Vis spectrophotometer (Fig.4b).The pH 9.0 showed high synthesize and good stability of AgNPs was selected for further studies. This observation was inaccordance with the previous studies made by [27][11] reported that there was a slow rate of formation and aggregation of AgNPs at acidic PH whereas, [34] reported observed that at basic pH there was a possibility of Ag^+ precipitating as AgOH. They also found that the optimum condition for the preparation of AgNPs using *Terminaliachebul a*was at pH7.0. [9]who have reported a pH 7.0 was optimum for the formation of AgNPs using *Acalyphaindica* leaf extract.

Fig.4a. Effect of different pH:

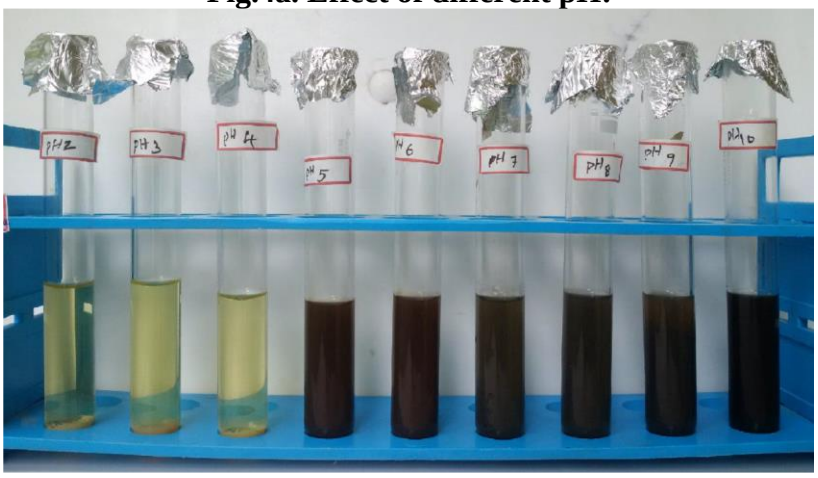
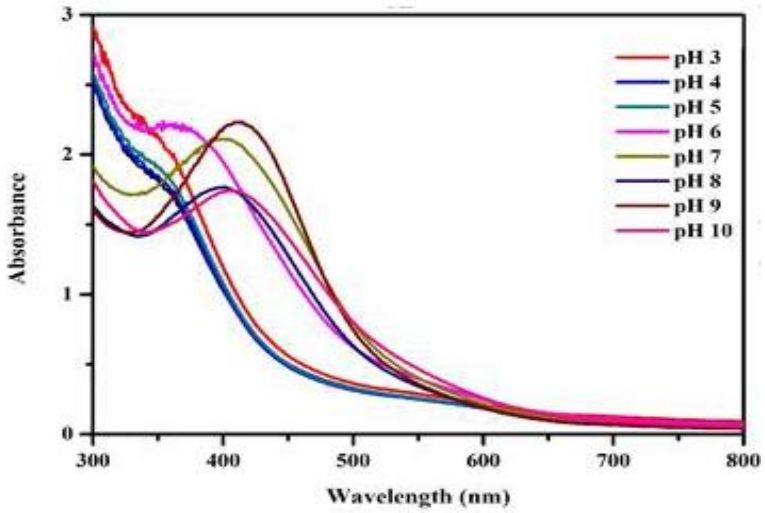


Fig.4b. UV-Vis spectrophotometer analysis



3.3.2. Effect of different concentration of silver nitrate:

The brown colour changes was observed (Fig.5a) in various concentration of silver nitrate from 100-500mM. The absorbance of the resulting solutions was measured UV-Visspectrophotometer (Fig.5b).Among them 0.1mM showed high synthesize of AgNPs and good stability was selected for further studies. These results were in agreement with the earlier investigations made by [21].[9][23] reported that 1.0mM silver ion concentration was found to be the best for the synthesis of AgNPs using *Annonasquamosa* peel extract.

Fig.5a. Effects of various silver nitrate concentration

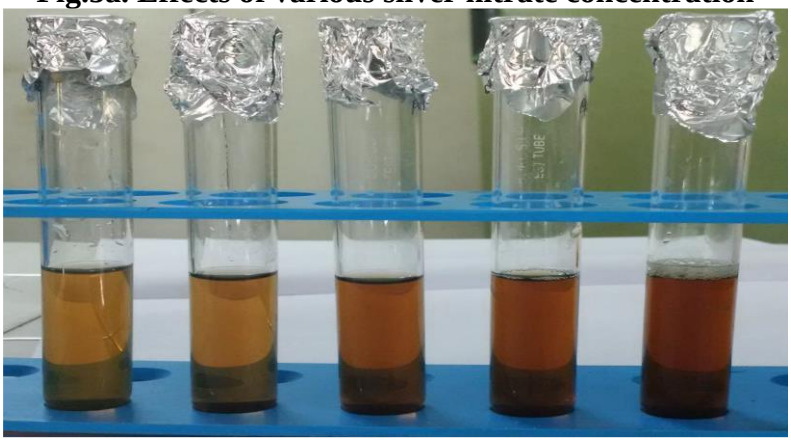
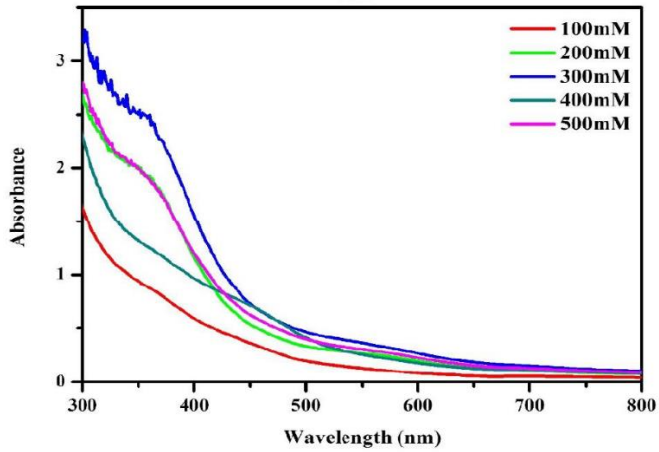


Fig.5b. UV-Vis spectra analysis at various silver nitrate concentration*:*



3.3.3. Effect of different concentration of substrate (flower extract):

The brown colour was observed (Fig.6a) in various substrate concentration from 0.5 to 2.5ml. The absorbance of the resulting solutions was measured UV-Vis spectrophotometer (Fig.6b). Among them 2.5 ml of flower extract added with 0.1mM solution supported maximum biosynthesize of nanoparticles. Therefore, this mixture was chosen for the following studies. The peaks corresponding to 1.5mL-3mL added slowly resulted a gradual shifting the peaks up to 450nm. However, 1.0mL of leaf extracts supported high stability than therest. [32] researchers reported that different concentration (0.5, 1.0, 1.8, 2.8, 3.8 and 4.8mL) of Tansy fruit used for the synthesis of AgNPs and AuNPs, and the concentration of 1.0mL was found suitable for the synthesis of nanoparticles.

Fig.6a. Effect of different concentration of substrate (flower extract)

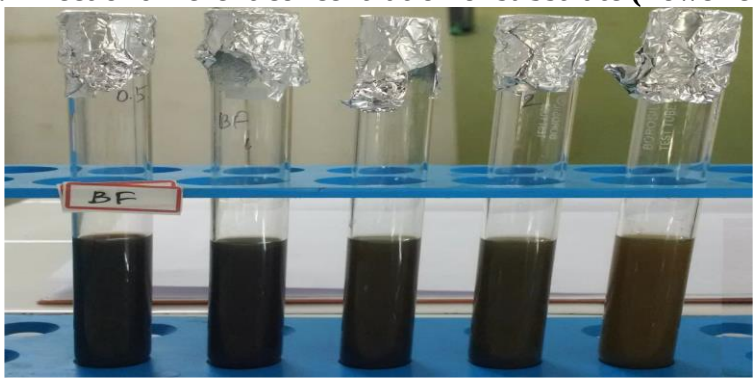
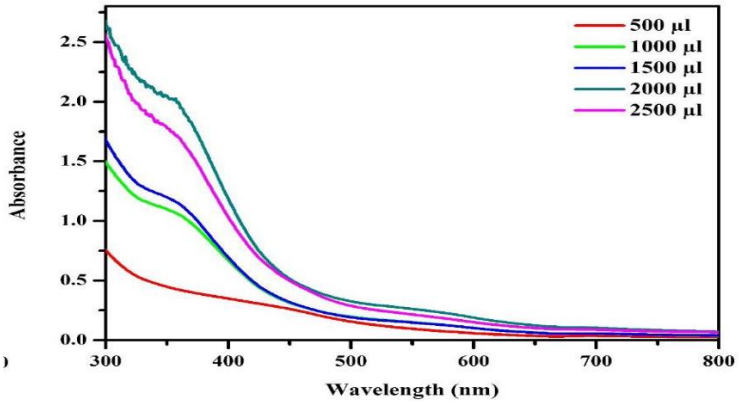


Fig.6b. UV-Vis spectra analysis at various substrate concentration (Flower extract)



3.4. Characterization of synthesized silver nanoparticles:

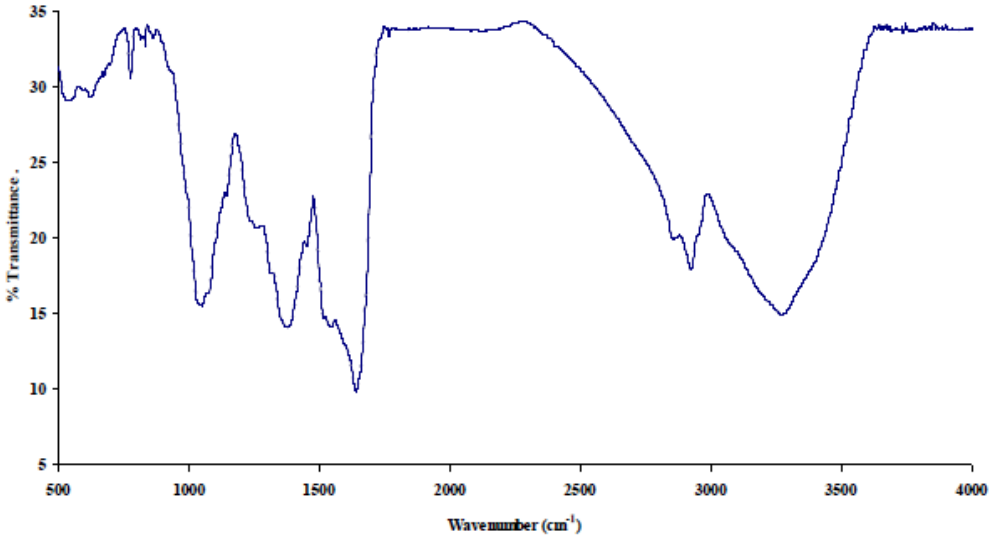
3.4.1. UV-Vis spectra analysis:

The AgNPs reduction in the aqueous solution of silver complex during the reaction with the flower extracts of *Cassia alata* was confirmed by the UV–Visspectra. The absorption maxima of AgNPs recorded after incubation. The absorption of reddish brown colour colloids showed notable 420 nm indicating presence of lone spherical AgNPs.

3.4.2. Fourier Transform Infra-Red (FTIR) Spectra analysis:

FTIR analysis was performed to identify the functional group of biomolecule to synthesis AgNPs to identify as the functional group (Fig. 7). The results of FTIR analysis of this study show different stretches of bonds shown at different peaks The band which appeared at 3465 cm^{-1} corresponded to H-OH stretching of phenols. The adsorption of band at 1635 cm^{-1} denotes the C=O stretching of aldehydes and ketones. The beak at 1678 cm^{-1} corresponding to N=O bending of nitro groups. The band observed at 1008cm^{-1} represent the C-O stretching of esters. The peaks observed at 845 cm−1 correspond to C–H stretching of alkenes

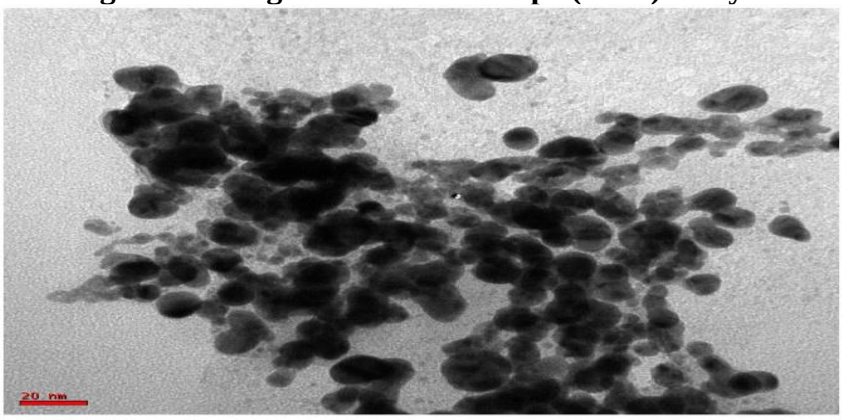
Fig.7. Fourier Transform Infra-Red (FTIR) Spectra analysis



3.4.3. Transmission Electron Microscope (TEM) analysis:

Morphology and particle size of AgNPs were characterized using TEM. The TEM images of AgNPs synthesized with the flower extract of *Cassia alata* are showd.The particles are predominantly spherical in shape with a diameter ranging from 20nm (Fig.8). [29]reported that the silver nanoparticles were spherical in shape in the range of 17–29nm.

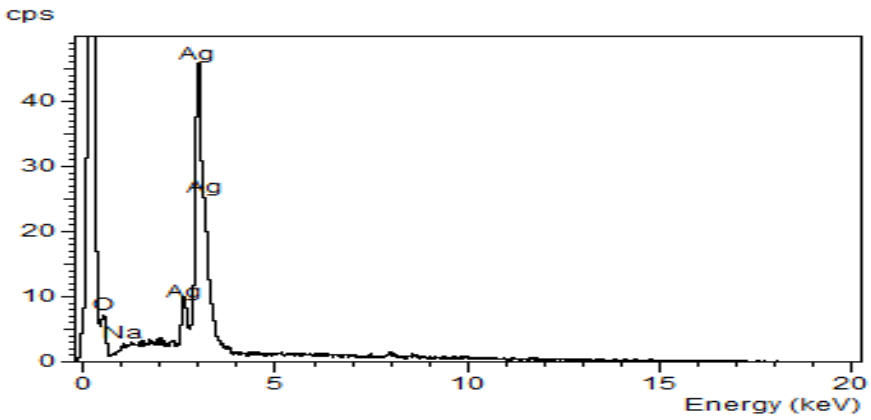
Fig.8. Scanning electron microscope (SEM) analysis



3.4.4. Energy dispersive analysis X-ray (EDX):

EDX spectrum shows (Fig. 9) peaks corresponding to the elements making up the true composition of the sample. The elemental profile of synthesized nanoparticles shows the highest x-ray energy peak at 3 keV due to silver and confirms the presence of silver. [5] similar the elemental profile of synthesized nanoparticles shows the highest x-ray energy peak at 3 keV due to silver and confirms the presence of silver.

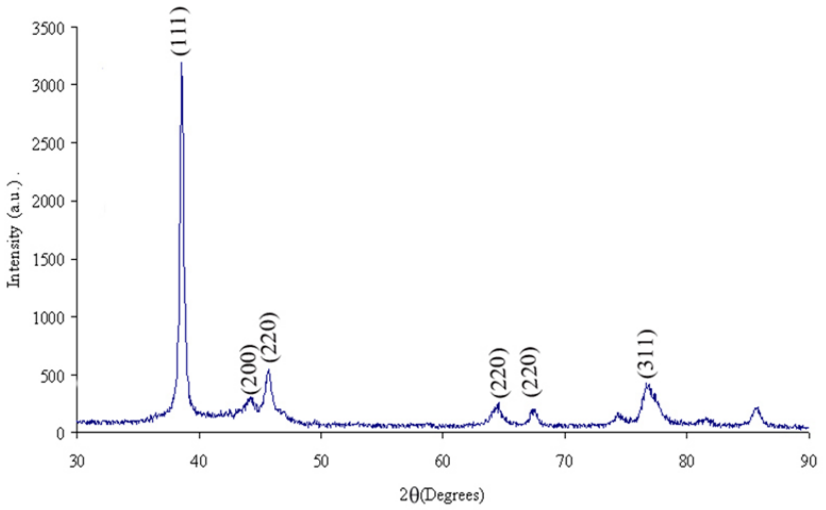
Fig.9. Energy dispersive analysis X-ray (EDX):



3.4.5. X-ray diffraction (XRD) analysis:

The crystalline nature of the green synthesized AgNPs by *Cassia alata* flower extract was also confirmed by x-ray diffraction patterns (XRD) analysis. Represents the XRD pattern gained for the synthesized AgNPs using the aqueous flower extract of *Cassia alata*. The size, phase identification and crystalline nature of the AgNPs were determined by the XRD analysis. It showed (Fig. 10) intense and sharp peaks at 2θ = 38.05°, 45.6°, 47.27°,66.2°, 68.9°, and 77.41°. XRD pattern showed four diffraction peaks 32.05°, 46.27°, 55.25°, and 57.39° can be indexed to the 111, 200, 220 and 311 planes of the face centered cubic crystalline silver respectively [5].The average sizeofAgNP calculated using Debye Schreyer equation by determining the full width at half maximum of the bragg's reflection corresponding to the (111) crystalline of AgNPs (Fig.10).The XRD results are consistent with those reported for face-centered cubic (fcc) lattice of silver [30].

Fig.10. X-ray diffraction (XRD) analysis



3.5. Antifungal activity of flower extract and synthesized silver nanoparticles:

3.5.1. Well Diffusion Method:

Antifungal effect of flower extract and AgNPs at the different concentration (50 and 100 µg) was quantitatively assessed on the basis of zone of inhibition (Table. 2 and 3). The maximum zone of inhibition ofwereobservedinAgNPswhich exhibited a good effect *Trichophytonrubrum* (28±0.89 mm) followed by *Xerophilicdermatitidis* (27±0.62 mm), *Aspergillus fumigates*(24±0.43mm), *Candida albicans* (22±0.67 mm) and *Candida immitis* (22±0.48 mm) (Fig. 11) and the zone of inhibition of flower extract against *Xerophilicdermatitidis* (21±1.23 mm) followed by *Candida immitis* (21±1.87 mm), *Xerophilicdermatitidis* (21±1.54 mm), *Candida albicans* (19±1.42 mm) and *Aspergillus fumigates* (18±1.45 mm) (Fig. 12). The zone of inhibition ofwere compared with standard antifungal agent nystatin.

Table.2. Antifungal activity of AgNPs:

Test pathogens	Concentration of leaf extract		Positive control (Nystatin)
	50µl	100µL	
	Zone of Inhibition in mm		
Aspergillus fumigates	19±0.98	24±0.43	14±0.23
Candida albicans	18±0.24	22±0.67	22±0.25
Candida immitis	20±0.35	22±0.48	22±0.23
Trichophyton rubrum	23±1.23	28±0.89	19±0.24
Xerophilic dermatitidis	22±0.43	27±0.62	13±0.25

Table.3. Antifungal activity of *C. alata* flower extract:

Test pathogens	Concentration of leaf extract		Positive control (Nystatin)
	50µl	100µL	
	Zone of Inhibition in mm		
Aspergillus fumigates	14±1.56	18±1.45	14±0.23
Candida albicans	14±1.32	19±1.42	22±0.25
Candida immitis	15±2.12	21±1.87	22±0.23
Trichophyton rubrum	15±2.32	21±1.23	19±0.24
Xerophilic dermatitidis	16±1.98	21±1.54	13±0.25

Fig.11. Antifungal activity of AgNPs:

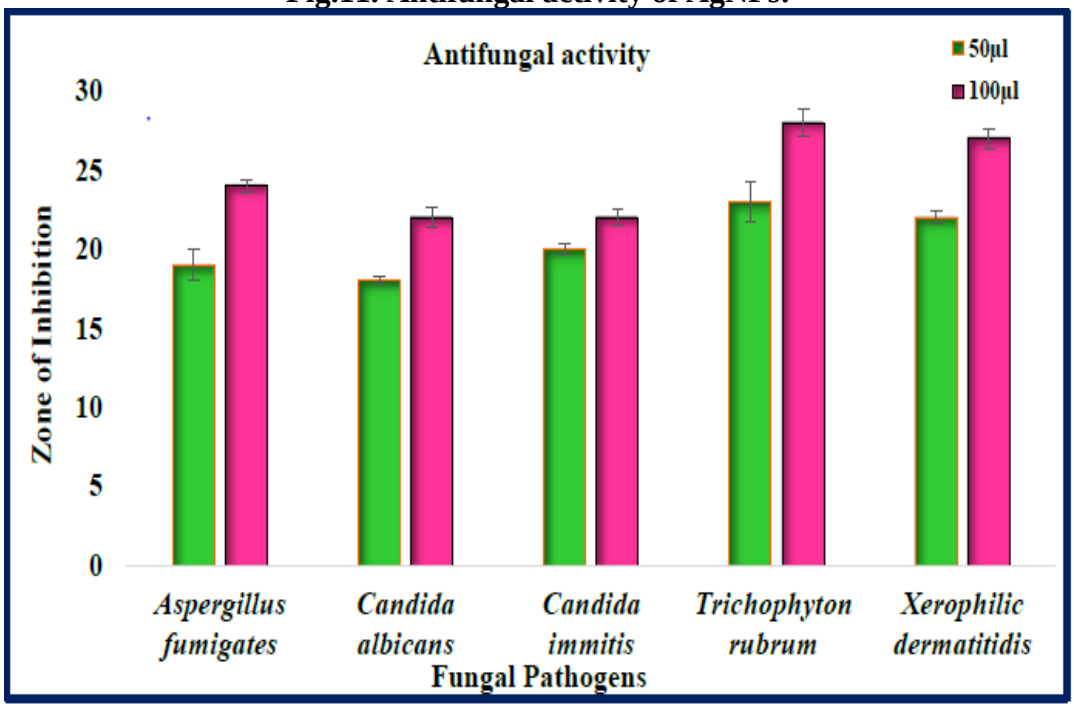
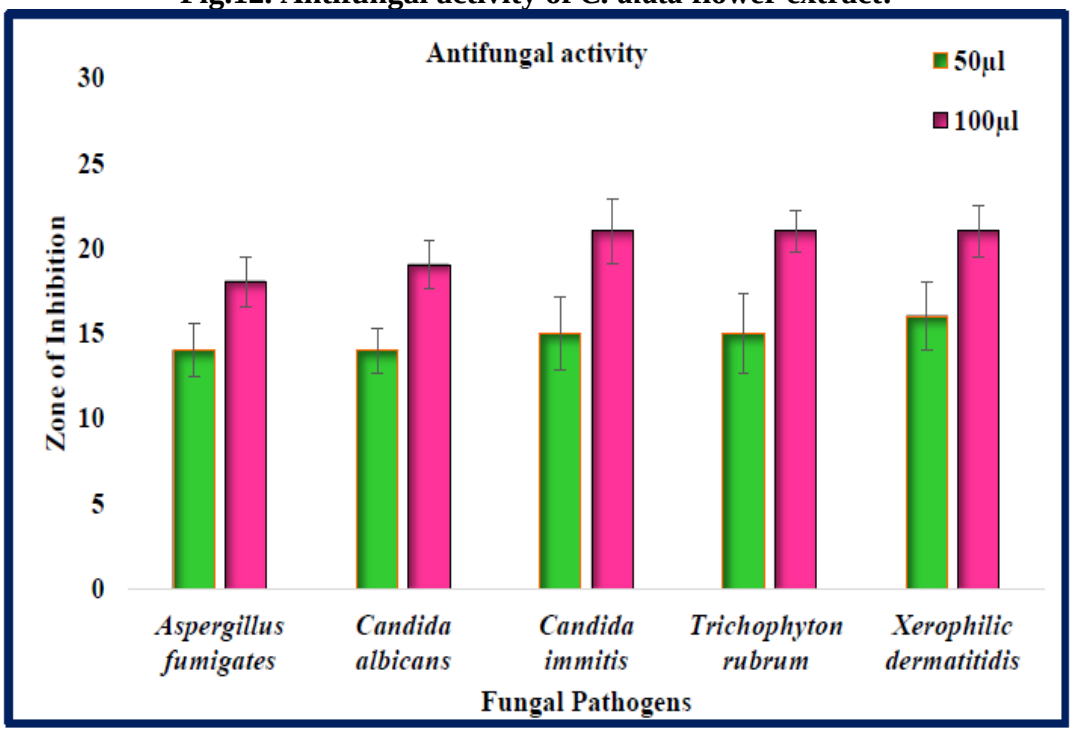


Fig.12. Antifungal activity of *C. alata* flower extract:



5.5.2. Minimum Fungicidal concentration

Minimum fungicidal concentration of flower extract was revealed by mean diameter of zone of inhibitions against dermatophytes. MFC values were recorded 20.0, 25.0 and 30.0, µl/ml against *Trichophytonrubrum Xerophilicdermatitidis* (27±0.62 mm), *Aspergillus fumigates* (24±0.43mm), *Candida albicans* (22±0.67 mm) and *Candida immitis* (22±0.48 mm) respectively.

3.6.Cell line

Based on the cell line result the low concentration of AgNPs was showed a 98% cell viability compare to other concentration show AgNPs 25µg/ml used for further study (Fig. 13 a & b).

Fig.13a. MTT assay using Human Fibroblast cell line L929

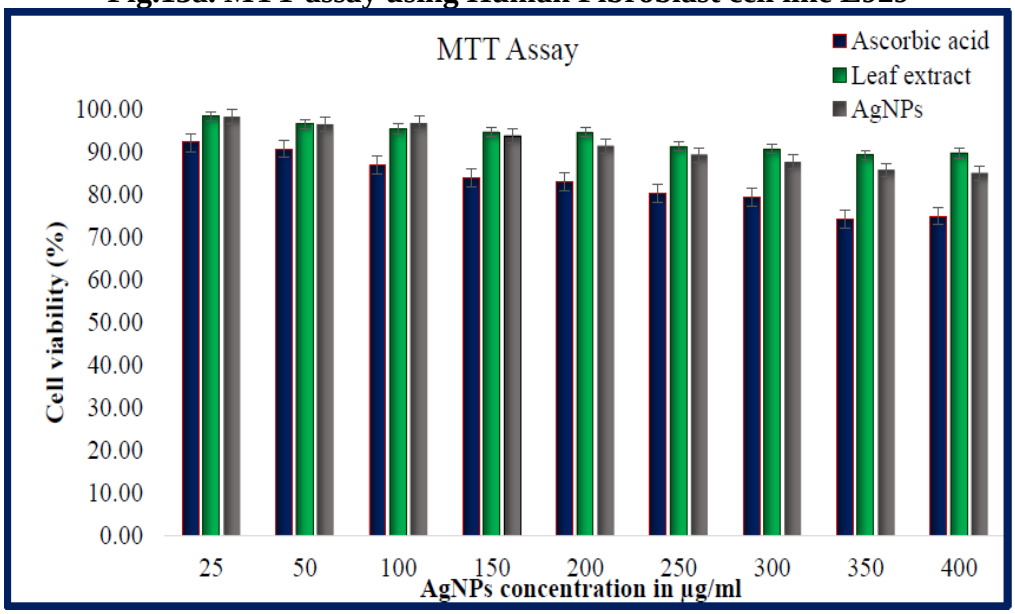
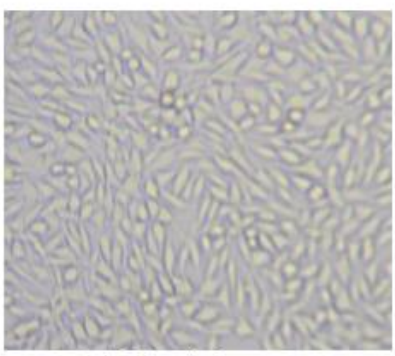
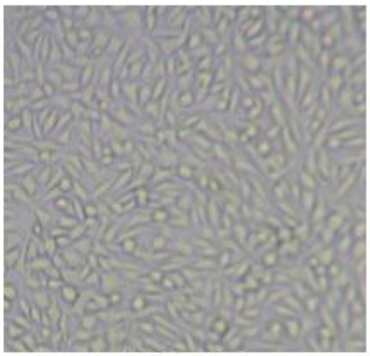


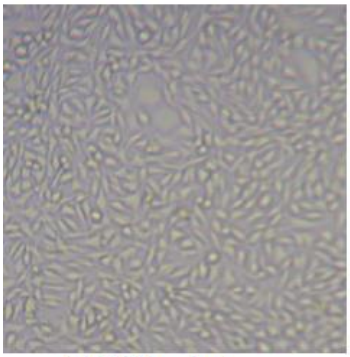
Fig. 13b. MTT assay using Human Fibroblast cell line L929 at various Concentration.



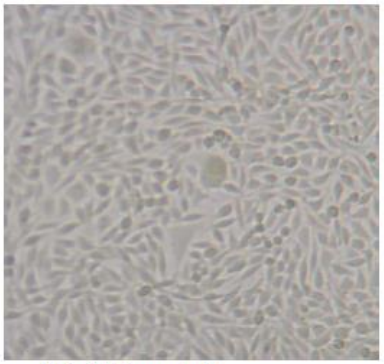
(a) 25 µg/ml



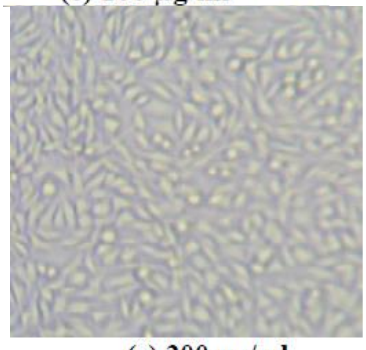
(b) 50 µg/ml



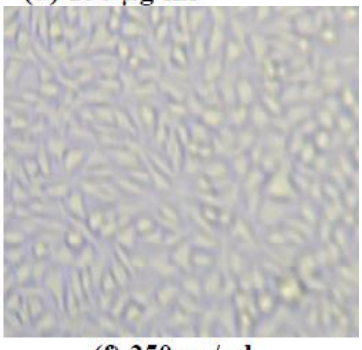
(c) 100 µg/ml



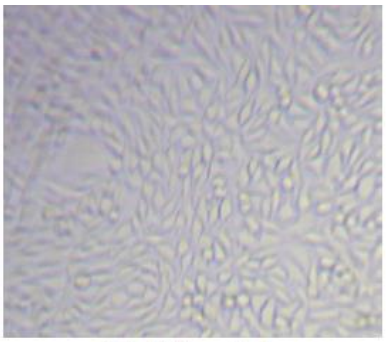
(d) 150 µg/ml



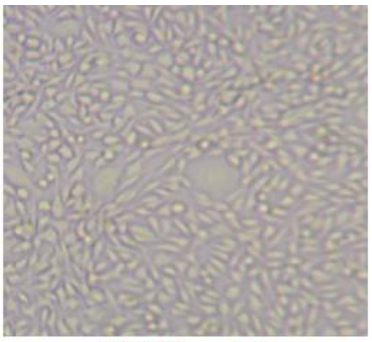
(e) 200 µg/ml



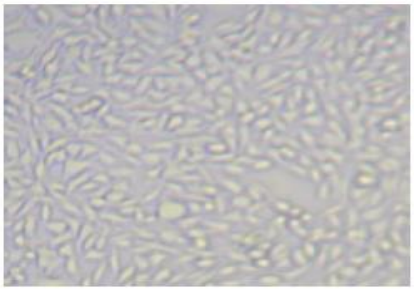
(f) 250 µg/ml



(g) 300 µg/ml



(h) 350 µg/ml



(i) 400 µg/ml

4. CONCLUSION

Biomedical remediation of skin disease using nano synthesized silver particle have easily accessible frequent fresh and economical yield growing technology applications. The antimicrobial materials containing various natural and inorganic substances have been intensive. Metal nanoparticles, while have a high specific surface area and a high fraction of surface atoms, have been studied extensively because of their unique physiochemical characteristics. Especially, silver nanoparticles from plant extracts play a vital role with promising antagonistic activities.

The medicinal plant *Cassia alata* were collected from Enathur village in Kanchipuram. Flower extracts was prepared by hot water extraction method. The reduction of silver nitrate into AgNPs during exposure to leaf extracts is followed by a gradual increase in colour development from clear to reddish brown, as a results surface plasmon resonance phenomenon. The absorption of reddish brown colour colloids showed notable 420 nm. Indicating presence of lone spherical AgNPs. In FTIR, the peaks of the spectrums of the aqueous extract of synthesized AgNPs, it contains AgNPs has occurred and new protein in the form of AgNPs have been formed study indicates that probably the amines, phenols and alkynes group in plant extract are involved in the reduction of Ag+ ionsAgNPs. Moreover, FTIR analysis also confirmed the presence of protein around the AgNPS acting as a reducing as well as stabilizing agent during synthesis of AgNPs. The TEM micrographs of AgNPs showed that they are spherical shaped, well distributed without aggregation in solution. The average size of the synthesised silver nanoparticles from *Cassia alata*was found to be 20 nm. The EDX spectrum peaks corresponding to elemental profile of synthesized nanoparticles shows the highest x-ray energy peak at 3 keV due to silver and confirms the presence of silver. The XRD pattern showed intense peaks (38.05°, 45.6°, 47.27°,66.2°, 68.9°, and 77.41°) in the whole spectrum of 2θ value ranging from 30 to 70. The typical XRD pattern revealed that the samples containing mixed phases (cubic, face- centered cubic) of silver nanoparticles in 2θ value (38.05°). AgNPs exhibited a good antifungal effect against *Trichophytonrubrum* (28±0.89 mm) followed by*Xerophilicdermatitidis* (27±0.62 mm), *Aspergillus fumigates* (24±0.43mm), *Candida albicans* (22±0.67 mm) and *Candida immitis* (22±0.48 mm)the zone of inhibition of respectively. Based on the cell line result the low concentration of AgNPs was showed a 98% cell viability compare to other concentration show AgNPs 25µg/ml used for further study

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Conflict Of Interest Statement
The authors declare no conflict of interest.

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