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A STUDY ON SYNTHESIS OF SILVER NANOPARTICLES AND ANTIBACTERIAL ACTIVITY OF *ACALYPHA HISPIDA*

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ABSTRACT

Most of the medicinal plants have antibacterial activity since they are rich in phytochemicals that possess drug producing properties. The current study carried out to perform the phytochemical analysis, antibacterial and silver synthesizing properties of *Acalypha hispida.* The leaves of the *Acalypha hispida* was collected, dried and treated with methanol and the methanol extract has been used for the above mentioned studies. The higher concentrations of methanol extract of *Acalypha hispida* inhibited the activities of microbes such as *K. pneumonia, P. aeruginosa, E. coli, S. typhi.* The phytochemical analysis of the methanol extract revealed that the plant has major phytochemicals except saponin, oils and resins and arthroquinone. It was also demonstrated that the plant (methanol extract) exhibits anti -bacterial activity and was determined by radial immunodiffusion techniques. The extract also has reducing capacity and reduced the silver nitrate solution to silver nanoparticles ($AgNP_s$). Silver nanoparticles are characterized by UV-Visible spectroscopy, FTIR and confirmed by SEM analysis. Silver nanoparticles play an important role in immunity development.

KEYWORDS: *Acalypha hispida*, Phytochemical analysis, Silver nanoparticles ($AgNP_s$), Antibacterial activity.

INTRODUCTION

Acalypha hispida, the chenille plant, is a flowering shrub which belongs to the family Euphorbiaceae, the subfamily Acalyphinae, and the genus Acalypha. Acalypha is the fourth largest genus of the Euphorbiaceae family, and contains many plants native to Hawaii and Oceania. This plant is also known as red hot cat's tail and fox tail in English.

Origin

The plant originated in tropical Asia and later spread to multiple countries in North America. In cultivation it is widespread, particularly as a houseplant and as an ornamental plant.

Description

It can grow up to 5–12feet (1.5–3.7m) tall, and have a spread of 3–6feet (0.91–1.83m), with potted plants being the smallest in growth. The plant has become somewhat domesticated, due to the nature and colour of its flowers. It can grow from seeds as well as from cuttings. It can be kept either as an outdoor plant or as a houseplant. *Acalypha hispida* is cultivated as a house plant because of its attractiveness and brilliantly coloured, furry flowers.

However, care should be taken in growing it, as all parts of the plant are poisonous. The plant is dioecious, and therefore there are distinct male and female members of the species. The female plant bears pistillate flowers which range in colour from purple to bright red, and grow in clusters along catkins. This feature is the primary reason the plant bears the nickname “red-hot cat tail”. The pistillates will grow all year long as long as the temperatures are favorable.

Hierarchy	**Description**
Kingdom	Plantae
Class	Angiosperms
Order	Malpighiales
Family	Euphorbiaceae
Genus	Acalypha
Species	A. hispida
Binomial name	*Acalypha hispida*

Silver Nanoparticles (AgNPs)

Nanoparticles have unique properties as a consequence of their size, distribution and morphology and, therefore, are a very important component in the rapidly developing field of nanotechonology. Silver has been known, for more than 2000 years, as a metal that exhibits good medicinal properties, silver- based compounds are being used in numerous antimicrobial applications.

Silver ions are highly toxic for microorganisms and, therefore, have multiple roles in the medical field AgNPs are a very important part of nanotechnology mainly because they do not induce modification on living cells and, therefore, are unable to cause microbial resistance. Recent studies revealed that AgNPs have the ability to attach to cell walls and alter cellular respiration. AgNPs are widely used in biology and medicine especially because of their attractive and unique physiochemical properties. Research carried out in the late 1970s used silver particles for the treatment of orthopedic diseases caused by different infections with microorganisms and faster bone recovery was noticed. Many other applications can be attributed to AgNPs, for example: catalysts in chemical reactions, bio-labeling, spectral selective coatings for the absorption of solar energy, food additives, textile industry etc. The current study is to analyse the pharmaceutical properties and anti-bacterial activity of Silver nitrate extract of *Acalypha hispida* leaves.

MATERIALS AND METHODS

Plant collection and preparation of extracts

The *A. hispida* leaves were colleted freshly from Yerkad forest near Salem and were shade dried for 5 days. The sun dry method was avoided, since it may have decreased the potency of ingredients present in the leaves. 10 gms of well dried and powdered leaves taken and boiled with 100ml methanol (100%) in Soxlet apparatus. The crude solution was filtered and used as extract for analysis.

Reagent preparation

Working stock solutions are prepared by diluting the stock solutions with double distilled water. Nutrient agar for bacteriological culture was obtained from Hi-media, Mumbai. Pure cultures of *K.pneumonia, P.aeruginosa, E.coli, S.typhi* were obtained from MTCC, Chandigarh.

Analysis of *Acalypha hispida*

Phytochemical Screening

Preliminary phytochemical analysis was carried out for the methanol extract of *Acalypha hispida* leaves as per standard methods described by Brain and Turner (1975) and Evans (1996).

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

Mayer's test

Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner's test

Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

H_2SO_4 test: Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

Detection of Steroids

Liebermann- Burchard test

2ml of acetic anhydride was added to 0.5g of the extract, each with 2ml of H_2SO_4. The colour changed from violet to blue or green in some samples which indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test

0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inner face indicates the presence of terpenoids.

Detection of Anthroquinones

Borntrager's test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink colour indicates the presence of anthraquinones.

Detection of Phenols

Ferric chloride test: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on a water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled in a water bath with 0.2ml each of Fehling solution A and B. A red precipitate indicates the presence of sugar.

Fehling's solution A: Copper sulphate (34.66g) is dissolved in distilled water and made up to 500ml using distilled water.

Fehling's solution B: Potassium sodium tartarate (173g) and sodium hydroxide (50g) is dissolved in water and made up to 500ml.

Detection of Oils and Resins

Spot test: Test solution was applied on filter paper. It developed a transparent appearance. It indicates the presence of oils and resins.

Synthesis of Silver Nano-Particles

The Silver nitrate solution (1mM solution) was prepared in 100ml flask. 1ml of plant extract was mixed with 9ml of 1mM of silver nitrate. The aqueous solution of the leaf extract and silver nitrate solution were used as a control throughout the experiment (Smetana et al., 2005). The final solution of 200 ml and centrifuged at 18,000rpm for 25min. The collected pellets were stored at - 4^0C. The supernatant was heated at 50^0 C to 95^0 C. A change in the colour of the solution was observed during the heating process.

Screening of Antibacterial activity: Three bacterial strains were used throughout the investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Preparation of inoculums: Stock cultures were maintained at 4^o C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton Broth (MHB) for bacteria that were incubated without agitation for 24hrs at 37^o C and 25^o C respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0 X 10^6 colony forming units (CFU/ml) for bacteria.

Antimicrobial susceptibility test: The disc diffusion method (Bauer et al, 1966) was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15ml of molten media into sterile petriplates. The plates were allowed to solidify for 5minutes and 0.1% inocula suspension was swabbed uniformly and the inocula were allowed to dry for 5minutes. The concentration of extracts of 40mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of the medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^o C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with a transparent ruler in millimeters.

RESULTS AND DISCUSSION

The test results of the present study are tabulated below and are compared with the previous studies on the selected species.

Phytochemical Analysis of *Acalypha hispida*:

Table. 1. Phytochemical Analysis.

Phytochemicals	Observations	Extracts of Methanol
Alkaloids		
Mayer's test Wagner's test	Cream colour Reddish brown solution/ precipitate	+ +
Flavonoids		
Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange colour precipitate	++ ++
Steroids		
Liebermann-Burchard test	Violet to blue or Green colour formation	+
Terpenoids		+
Salkowski test	Reddish brown precipitate	
Arthroquinone		
Borntrager's test	Pink colour	-
Phenols		
Ferric chloride test Lead acetate test	Deep blue to Black colour formation White precipitate	+- +-
Saponin	Stable persistant	-
Tannin	Brownish green / Blue black	+
Carbohydrates	Yellow / brownish / blue / green colour	+
Oils & Resins	Filter paper method	-

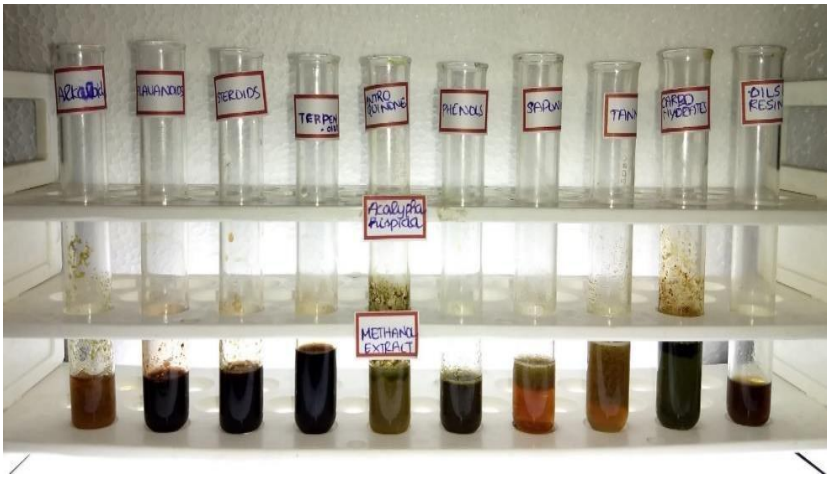

Figure. 1: *Acalypha hispida* Phytochemical analysis.

Antibacterial activity of *Acalypha hispida*

Table. 2: Antibacterial activity and Results.

Sr.No	Bacteria	Control (Inhibition zone) (mm)	Concentration(mg/µl)			
			40	50	60	70
1.	*K.pneumonia*	26	11	12	14	19
2.	*P.aeruginosa*	37	12	14	16	16
3.	*E.coli*	25	12	13	15	19
4.	*S.typhi*	26	10	12	17	21

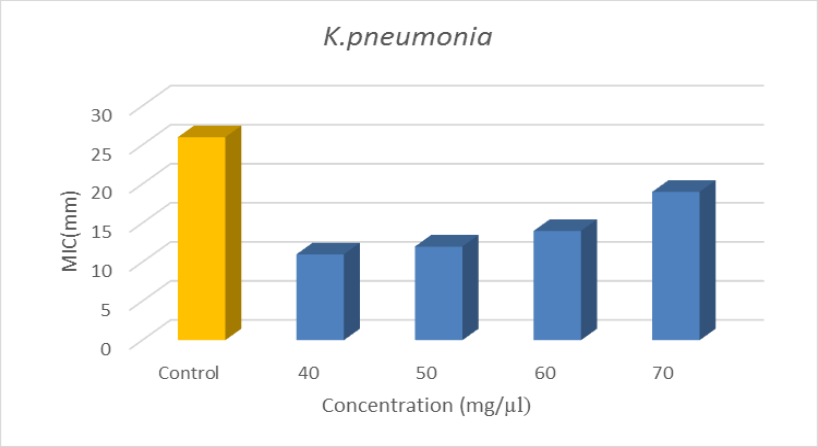

Figure. 2: Antibacterial activity of *Acalypha hispida* methanolic extract against *K.pneumonia.*

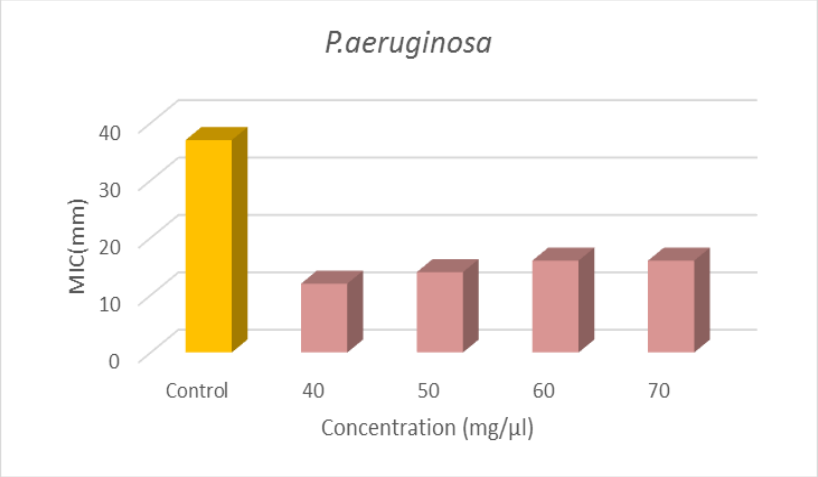

Figure. 3: Antibacterial activity of *Acalypha hispida* methanolic extract against *P.aeruginosa.*

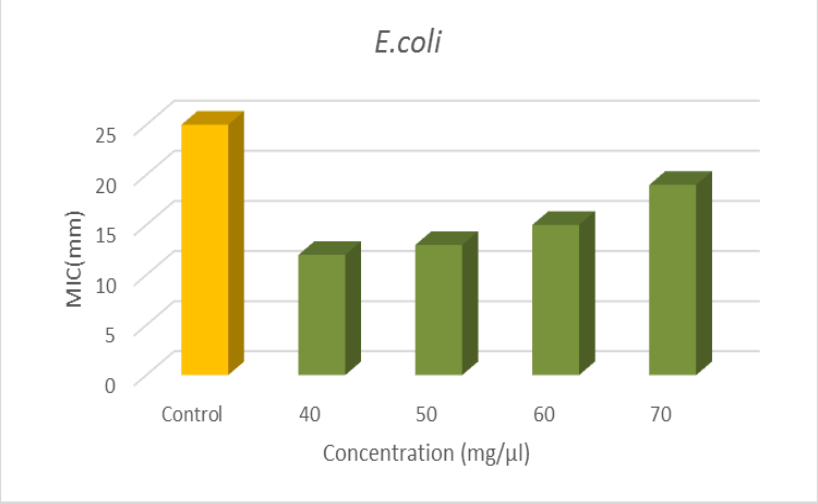

Figure. 4: Antibacterial activity of *Acalypha hispida* methanolic extract against *E.coli.*

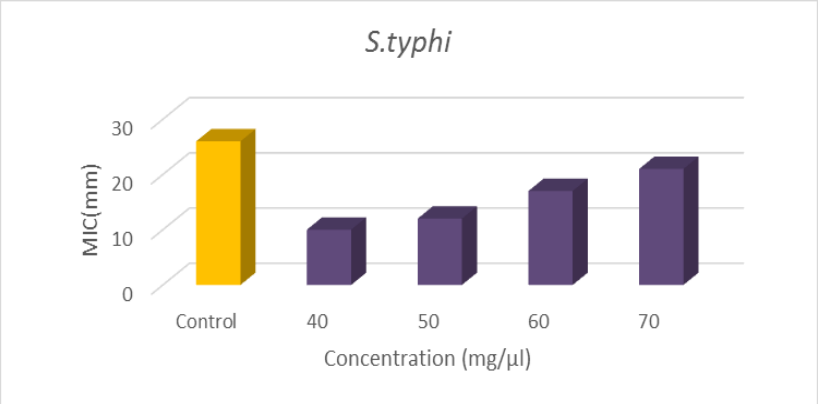

Figure. 5: Antibacterial activity of *Acalypha hispida* methanolic extract against *S.typhi*

Characterization of Silver Nanoparticles

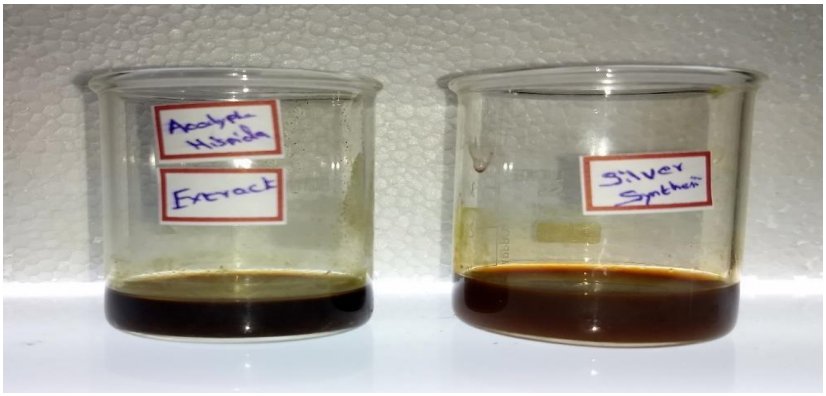

Fig. 6. Silver synthesis of *Acalypha hispida.*

UV-Vis Analysis

The optical property of Silver nanoparticles was determined by UV-Vis spectrophotometer (Perklin- Elmer, Lamda 35, Germany). After the addition of synthesized extract, the spectra's were taken in different time intervals up to 24hrs between 350nm and 500nm. Then the spectra taken after 24hrs of Silver were synthesized.

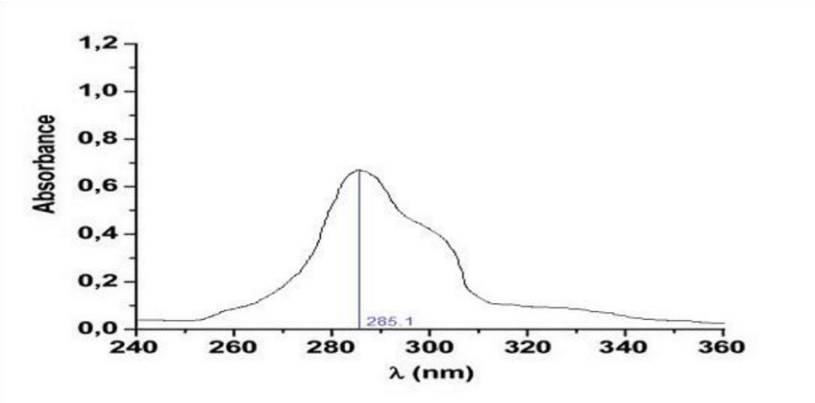

Fig. 7. UV spectrum.

FT-IR analysis

The chemical composition of the synthesized Silver nanoparticles was studied by using FT-IR spectrometer (Perkin-Elmer LS-55- Luminescence spectrometer). The solution were dried at 75^{o} C and the Silver synthesized was characterized in the range 4000–400cm^{-1}using KBr pellet method.

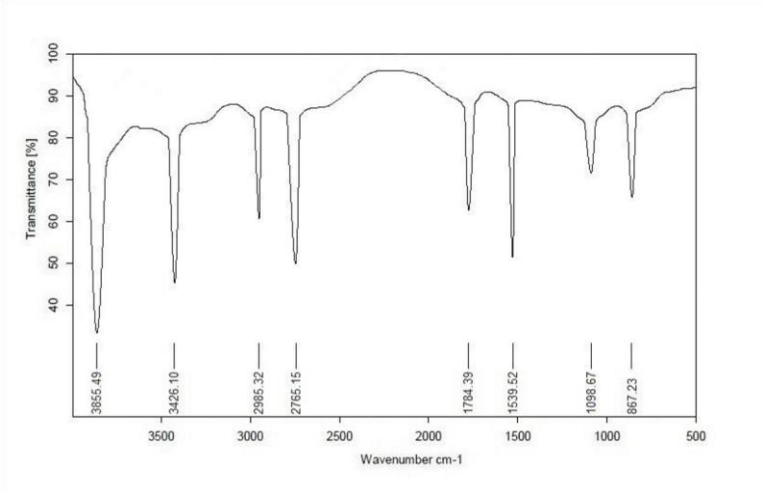

Fig. 8. FTIR spectrum.

SEM Analysis

The morphological features of synthesized silver nanoparticles from plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24 hrs of the addition of $AgNO_3$ the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20KV.

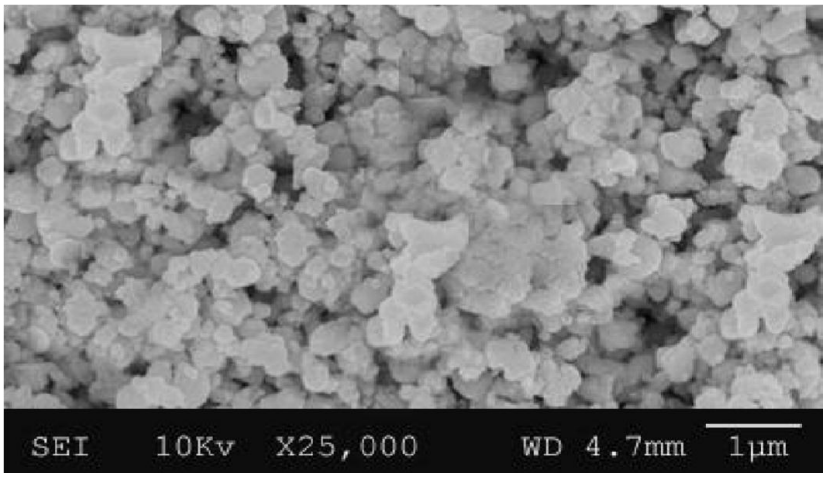

Fig. 9: SEM analysis.

CONCLUSION

Phytochemical screening of *Acalypha hispida* showed that it has sufficient phytochemicals needed for the medicinal properties for drug productions. Methanol extract of the leaves of the *Acalypha hispida* was subjected to phytochemical analysis and it reveals the presence of phytochemicals which are rich in medicinal properties and it can be used for drug production. (Rajput *et al* (2011)) The presence of phytochemicals was confirmed by Immuno diffusion technique. Ampicillin was used as control for disc diffusion method At 40, 50, 60, 70 (mg/ml) concentration, this methanol extract exhibited antibacterial activity against *K.pneumonia, P.aeruginosa, E.coli and S.typhi.* This study estimated that 70(mg/ml) is the suitable concentration for inhibiting the bacterial species. At 70(mg/ml) *S.typhi has* been inhibited with a zone formation of about 21mm diameter. *K.pneumonia and P.aeruginosa* has been inhibited with a zone formation of about 19mm diameter. *E.coli* has been inhibited with a zone formation of about 16mm diameter. The synthesis of silver nanoparticles from aqueous methanol plant extracts has huge advantages over other biological entities especially because they do not employ cell cultures. Although there are numerous literature reports regarding the green synthesis of silver nanoparticles still lot of plant extracts are being studied as research as potential candidates for the biosynthesis of silver nanoparticles.

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