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Preparation, Characterization and Evaluation of Silver Nanoparticles of Lilium candidum and Its Antimicrobial Activity

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ABSTRACT

Researchers are becoming more interested in green nanotechnology in the production of nanoparticles. Because of their biological and physicochemical characteristics, nanotechnologies find use in a wide range of industries, such as drug delivery, sensors, optoelectronics, and magnetic devices. Green synthesis is an environmentally benign method that should be investigated further to see if various plants can produce nanoparticles. Silver nanoparticles range in size from one to one hundred nanometres. Since bacteria are becoming more and more resistant to many antibiotics and because excessive use of these synthetic treatments can be harmful and have further negative effects on the human body, multidrug resistance has emerged as a significant and difficult problem for the pharmaceutical industry. The current study assesses the antibacterial, antioxidant, and phytochemical screening properties as well as the green production and antimicrobial activity of silver nanoparticles. Crude extracts of *Lilium candidum* leaves were made using three different solvents: petroleum ether, ethyl acetate, and methanol. Using the reducing power assay technique and DPPH, antioxidant activity was investigated. Silver nanoparticles were created by combining *Lilium candidum* leaf aqueous extract with a 1 mM AgNO3 solution. FTIR spectroscopy and UV-Vis spectrometry were used to characterise the produced silver nanoparticles. The agar well diffusion method was used to investigate antimicrobial activity. Several phytochemicals, including flavonoids, alkaloids, saponins, carbohydrates, terpenoids, steroids, tannins, and free anthraquinones, were found to be present in the methanol extract, according to the results of the phytochemical screening. The leaves of the methanolic extract had a total phenolic content of 0.068 mg/gm, with flavonoids coming in second at 1.089 mg/gm. Similarly, methanol extract demonstrated concentration-dependent antibacterial and efficient free radical scavenging properties. The generated silver nanoparticles characterisation results showed that plant phyto-constituents create and stabilise the silver nanoparticles, and they also demonstrated positive antibacterial properties. The use of natural resources is the best option for producing NPs as a sustainable, environmentally friendly, cost-effective, and chemically contaminant-free method. The green synthesis process is a crucial field in nanotechnology. There are numerous possible biological and medicinal uses for these silver nanoparticles.

Keywords: *Lilium candidum, Phytochemical screening, Antioxidant activity, Silver nanoparticles, FTIR spectroscopy, Antimicrobial activity.*

1. INTRODUCTION

I In order to control microbial illnesses, new warfare strategies must be investigated in order to combat germs that are resistant to many drugs and to curb the issue of chemical drug intake. These plant-based medications are less dangerous, have few adverse effects, and are reasonably priced[1]. In traditional herbal medicine, plants and their components roots, stems, bark, leaves, flowers, fruits, seeds, and exudates are a key component of medications. The utilisation of appropriate and genuine raw materials is crucial to the therapeutic efficacy of the medications utilised in these systems[2]. In order to control microbial illnesses, new warfare strategies must be investigated in order to combat germs that are resistant to many drugs and to curb the issue of chemical drug intake. These plant-based medications are less dangerous, have few adverse effects, and are reasonably priced1. In traditional herbal medicine, plants and their components roots, stems, bark, leaves, flowers, fruits, seeds, and exudates are a key component of medications. The utilisation of appropriate and genuine raw materials is crucial

to the therapeutic efficacy of the medications utilised in these systems[2]. To control microbial diseases, new warfare strategies that target drug-resistant bacteria and lessen the issue of chemical drug intake must be investigated. These plant-based medications are less expensive, less dangerous, and have fewer adverse effects1. In traditional herbal therapy, the roots, stems, bark, leaves, flowers, fruits, seeds, and exudates of plants are all vital components. The utilisation of appropriate and genuine raw materials is essential to the therapeutic efficacy of the medications utilised in these systems[2]. Numerous medicinal plants are major sources of antibiotics, according to research that tests their extracts and products for antibacterial and antioxidant qualities.[3]. Native American tribes have utilised medicinal herbs for their own purposes.[4]. Antioxidant effects are crucial in the management of human diseases. The majority of pathogenesis is caused by reactive oxygen species (ROS) associated with lipid peroxidation.[5]. Free radicals are scavenged by antioxidants, which fight oxidative stress. One of the most important characteristics of plant extracts is their antioxidant activity, as researchers have searched for natural antioxidant sources to incorporate into numerous food, medicine, and cosmetic compositions. The synthesis of nanomaterials is currently one of the most active areas in nanoscience. Special attention has been dedicated to nanomaterials that help improve the human quality life. A remarkable example is the silver nanoparticles silver nanoparticles which are known by their inhibitory and bactericidal effects. Silver nanoparticles can be produced with various sizes and shapes depending on the fabrication method which can be physical, chemical, biological and hybrid. The chemical methods use toxic chemicals, which are not friendly to environment making them unsuitable for biomedical applications. Specifically, the widely used chemical reduction methods[6] usually employ toxic and perilous chemicals that are responsible for various biological risks. On the other hand, physical methods are expensive and incompatible with sizeable production of nanoparticles. Consequently, green synthesis techniques have emerged to circumvent poisonous and dangerous chemicals, garnering considerable attention due to their environmental friendliness, speed, ease of use, and energy efficiency[7]. Due to the abundance of biomolecules with the capacity for both bioreduction and biostabilization, green synthesis employing large biological molecules extracted from plant extracts[8] could make it easier to control the size and shape of metal nanoparticles. In particular, a variety of plants have been utilised to synthesise silver nanoparticles[9],[10], including Acacia nilotica pod extract[11], Terminalia cuneata, Illicium verum (star anise), and the stem bark of Callicarpa maingayi. The so-called white Madonna lily, *Lilium candidum* L. (Liliaceae), is well-known in traditional medicine and includes a number of physiologically active substances. This plant's blooms and bulbs have been used to treat burns, wounds, inflamed skin, finger ulcers, furuncles, and ulcers[12]. *L. candidum* is extremely vulnerable to fungus and plant viruses. [13]. For medicinal, decorative, and fragrance purposes, this plant is successfully grown in Europe, the United States, and numerous other nations, including Iraq[14–16]. Alkaloids such as pyrroline and pyrrolidine, on the other hand, may be involved in causing substantial oxidative stress and DNA damage, which results in necrosis or cell death[17]. Because of the plant's immense pharmacological and ethnobotanical benefits, the current study aimed to identify the qualitative and quantitative features of the phytochemical constituents as well as the antibacterial and antioxidant qualities of *Lilium candidum* leaf extracts. Attempts were also undertaken to characterise and evaluate the antibacterial qualities of these plant leaf-mediated silver nanoparticles, as there hasn't been a prior publication on the environmentally friendly creation of *Lilium candidum* leaf silver nanoparticles.

2. MATERIALS AND METHODS

Plant material

Lilium candidum leaves were gathered from the Bhopal (M.P.) neighbourhood. Senior botanist Dr. Zia-Ul-Hassan, professor and director of the botany department at Safia College of Arts and Science, Peer Gate Bhopal, identified the sample. A plant herbarium was delivered to Safia College of Arts and Science's specimen library, peer gate Bhopal, and *Lilium candidum's* specimen voucher number is 119/Bot/Saf/49.

Chemical reagents

For this investigation, Sigma-Aldrich Chemicals is the supplier of silver nitrate (AgNO3). We bought dimethyl sulfoxide (DMSO) from Merck in India. We bought the pH buffer tablets from Himedia. From Himedia Laboratories in Mumbai, India, we acquired Nutrient Agar, Nutrient Broth, Agar Agar, Muller Hinton Agar (MHA), and Sabouraud Dextrose Agar Media. Triple-distilled water was used to create the aqueous solutions. Every chemical employed in this investigation was of analytical quality.

Extraction of plant material

Cold maceration

Leaves of *Lilium candidum* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. About 500gm of the leaves powder was macerated with pet. ether, ethyl acetate and methanol and stored for 72 hours in ice cold condition for the extraction of phytochemicals. At the end of the third day extract was filtered using whatmann No. 1 filter paper to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[18]. For extraction of aqueous extract, 250g of p owdered plant material was soaked in 450mL

of water and kept at room temperature for 48 hours with periodical shaking. The solvent extract was filtered through muslin cloth and collected in a beaker and then filtrate was place on water bath for solvent evaporation. Finally browinesh black, solid crude aqueous leaf extract of plant obtained.

Total phenolic content estimation

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Concentration of (20-100 µg/ml) of gallic acid was prepared in methanol. Concentration of 100 µg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2 ml of a 10-fold dilute folin Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. The tubes were covered with para film and it was then incubated at room temperature for 30 min with intermittent shaking and the absorbance were taken at 765 nm against using methanol as blank. Total phenolic content was calculated by the standard regression curve of gallic acid and the results were expressed as gallic acid equivalent (mg/g) [19].

Total flavonoid content estimation

Different concentration of rutin (20 to 100µg/ml) was prepared in methanol. Test sample of near about same polarity (100µg/ml) were prepared. An aliquot 0.5ml of diluted sample was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% $NaNO_2$ solution. After 6 min, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 5min, and then 2 ml of 4% NaOH solution was added to the mixture. The final volume was adjusted to 5ml with distilled water and allowed to stand for another 15 min. Absorbance was determined at 510 nm against water as blank. Total flavonoid content was calculated by the Standard regression curve of Rutin/ Quercetin[20].

Antioxidant activity

DPPH radical scavenging activity

For DPPH assay, the method of Gulçin *et al.,* 2006[21] was adopted. A solution of 0.1mM DPPH (4mg/100ml) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of different concentrations of the different extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid was used as reference standard while methanol was used as control. Reduction of the stable DPPH radical was used as a marker of antioxidant capacity of *Lilium candidum* extracts. The change in colour was measured at 517 nm wavelength using methanolic solution as a reference solution. This was related to the absorbance of the control without the plant extracts. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. All the tests were carried out in triplicates. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

Reducing power assay

A spectrophotometric method was used for the measurement of reducing power. For this 0.5 ml of each of the extracts was mixed with 0.5ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide (10 mg/ml). The reaction mixture was incubated at 50ºC for 20 min separately and then rapidly cooled, mixed with 1.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (0.5ml) of the supernatant was diluted with distilled water (0.5ml) and then ferric chloride (0.5ml, 0.1%) was added and allowed to stand for 10 min. the absorbance was read spectrophotometrically at 700 nm. Ascorbic acid (AA) was used as standard for construction of calibration curve[22].

Reducing Power (%) = (As / Ac) × 100

Here, Ac is the absorbance of control (AA) and as is the absorbance of samples (extracts) or standards.

Green synthesis of silver nanoparticles

100 ml of 1mM aqueous solution of $AgNO_3$ solution was taken in Erlenmeyer flask and 15 ml of aqueous stem extract was added drop wise into it for bio reduction process at room temperature. The reaction mixture was allowed to stir at 200 rpm using magnetic stirrer till the solution was turned from yellow to dark brown indicating the formation of silver nanoparticles. The reduced solution was centrifuged at 5000 rpm for 30 min to get clear supernatant. The supernatant was discarded and the particles obtained were centrifuged with water repeatedly to get pure nanoparticles.

Characterization of silver nanoparticles

UV-spectrophotometer analysis

The synthesized silver nanoparticles formulation F3 (solution of 1 mg/ml in distilled water as a dispersive medium) were monitored using UV-Vis spectrophotometer (Systronics double beam spectrophotometer 2202, India) between the range of 200nmand800nm. Distilled water was used as blank for UV-vis Spectrophotometer analysis.

Fourier transforms infra red spectroscopy (FTIR)

The infrared spectra for the plant extract and synthesized silver nanoparticles were attained for the identification of functional groups in a (Perkin Elmer Spectrum 2, Germany) spectrophotometer IR affinity-1 by employing KBr pellet technique and registering amplitude waves ranging from 400 to 4000 cm−1.

Antibacterial activity study

Antibacterial study of different extract was carried out using agar well diffusion assay against selected bacterial strains. Prepared silver nanoparticles were diluted to the concentration of 50, 100, 200 and 250 mg/ml utilizing DMSO as the solvent. Nutrient agar media was prepared. Microbial strains grown in nutrient media were used. Microbial suspension of density 10^8 CFU/ml were used for inoculation on the Nutrient agar media. Four wells of 6 mm diameter and 5 mm depth were prepared on the solid agar in each plate using a sterile borer. Different concentrations of extracts, positive control (ofloxacin 10 µg/ml) and negative control (DMSO) were dispensed (50 µl). The plates were allowed to stand for 1 h at room temperature for diffusion of the extract and incubated at 37°C for 24 h. After 24 h, the zones of inhibition were measured using a digital Verniercaliper. All experiments were conducted in triplicate and the mean values of the diameter of inhibition zones ± standard deviations were calculated.

Antifungal activity

Antifungal study of prepared silver nanoparticles was carried out using well diffusion assay. silver nanoparticles were diluted to the concentration of 50, 100, 200 and 250 mg/ml utilizing DMSO as the solvent. Sabouraud dextrose agar media was prepared. Fungal strains (*A. niger* and *C. krusei*) grown in Sabouraud broth were used. Microbial suspension of density 10^8 CFU/ml were used for inoculation on the Sabouraud dextrose agar media. Four wells of 6 mm diameter and 5 mm depth were prepared on the solid agar in each plate using a sterile borer. Different concentrations of extracts, positive control (Amphotericin B 10 µg/ml) and negative control (DMSO) were dispensed (50 µl). The plates were allowed to stand for 1h at room temperature for diffusion of the extract and incubated at 28°C for 48 h. After 48 h, the zones of inhibition were measured using a digital Verniercaliper. All experiments were conducted in triplicate and the mean values of the diameter of inhibition zones ± standard deviations were calculated.

3. RESULTS AND DISCUSSIONS

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the leaves of the plants using petroleum ether, ethyl acetate and methanol as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder of leaves of *Lilium candidum* are shown in Table 2. Methanolic extracts of leaves sample of *Lilium candidum* showed the presence of alkaloids, flavonoids, phenols, tannins and glycosides but in Pet ether and ethyl acetate extracts all phytoconstituents was absents, only carbohydrates, alkaloids presents.

Table 1 Results of percentage yield of leaves extracts

Plant Name	**Percentage yield (%)**		
	Pet. Ether	**Ethyl acetate**	**Methanol**
Lilium candidum	3.3	4,6	8.4

Table 2 Phytochemical evaluation of *Lilium candidum* leaves

Tests	**Pet ether**	**Ethyl acetate**	**Methanol**
Carbohydrates			
Molish	+ ve	- ve	+ ve
Fehlings	+ ve	- ve	+ ve
Benedit's	+ ve	- ve	+ ve
Protien & amino acids			
Biurets	- ve	- ve	+ ve
Ninhydrin	- ve	- ve	+ ve

Glycosides			
Borntrager	- ve	+ ve	+ ve
Killer killani	- ve	+ ve	+ ve
Alkaloids			
Mayers	+ ve	- ve	+ ve
Hagers	+ ve	- ve	+ ve
Wagners	- ve	- ve	+ ve
Saponins			
Froth	- ve	- ve	+ ve
Flavonoids			
Lead acetate	- ve	- ve	+ ve
Alkaline reagent test	- ve	+ ve	+ ve
Treterpenoids & Steroids			
Salwoski	+ ve	- ve	+ ve
Libberman Burchard	+ ve	+ ve	+ ve
Tannin & Phenolics			
Ferric chloride	- ve	- ve	+ ve
Lead acetate	- ve	- ve	+ ve
Gelatin	+ ve	- ve	+ve

Quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoid content (TFC). The TPC was calculated with respect to gallic acid (standard) and TFC was then calculated with respect to rutin taken as standard. The TPC and TFC in methanolic extract were found to be 0.058mg/gm and 1.080 mg/gm respectively table 3& fig 1, 2.

Table 3 Total phenolic and flavonoid content of extracts

Test	**Methanolic extract**
TPC	0.059 mg/gm equivalent to Gallic acid
TFC	1.089 mg/gm equivalent to Rutin

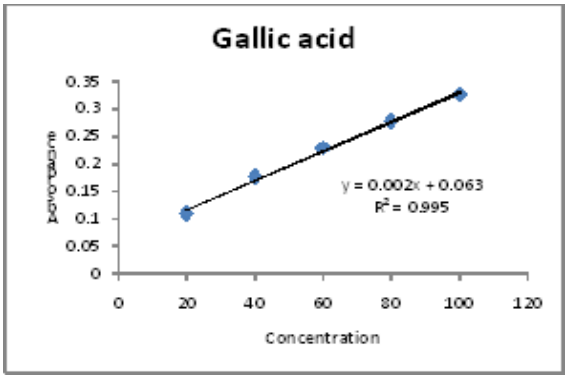

Fig. 1 Graph of estimation of total phenolic content

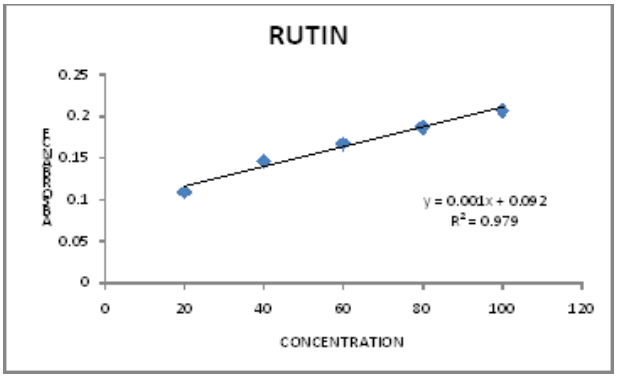

Fig. 2 Graph of estimation of total flavonoids content

Antioxidant activity of the samples was calculated through DPPH assay and reducing power assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard in both the tests and the values were comparable with concentration ranging from 20µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed. % inhibition was higher in the ascorbic acid while the values were lesser in methanolic extract table 4. The reducing ability of the compound usually depends on the reductants, which have been exhibited antioxidative capacity by breaking the free radical chain, donating a hydrogen atom. Reducing power assay was calculated in extracts and the values indicated a superior activity table 5& fig 3

Table 4 DPPH assay of ascorbic acid and methanolic extract

S. No.	Conc. (µg/ml)	Ascorbic acid (% Inhibition)	Methanolic Extract (% Inhibition)
1.	**20**	52.74123	47.80702
2.	**40**	56.35965	56.4693
3.	**60**	61.51316	65.57018
4.	**80**	68.9693	68.85965
5.	**100**	71.71053	74.01316
IC 50 Value		11.54	21.29

Table 5 Result of reducing power assay

S. No.	Conc. (µg/ml)	Ascorbic acid	Methanolic Extract
1.	**20**	0.987	0.533
2.	**40**	1.032	0.712
3.	**60**	1.145	0.716
4.	**80**	1.159	0.762
5.	**100**	1.196	0.800

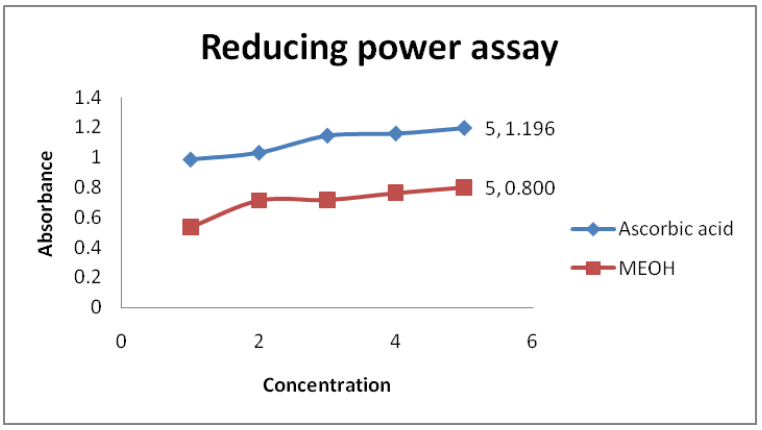

Fig 3 Reducing power assay

Synthesis of the silver nanoparticles in aqueous solution was checked by recording the absorption spectrum at a wavelength range of 200-700 nm. The UV-Vis absorption spectrum of silver nanoparticles Fig. 4 obtained showed the absorption maxima ranged from 408-442 nm which gives the confirmation for the silver nanoparticles synthesis. FTIR spectra of *Lilium candidum* leaf extract and synthesized silver nanoparticles are shown in Fig 4-6.

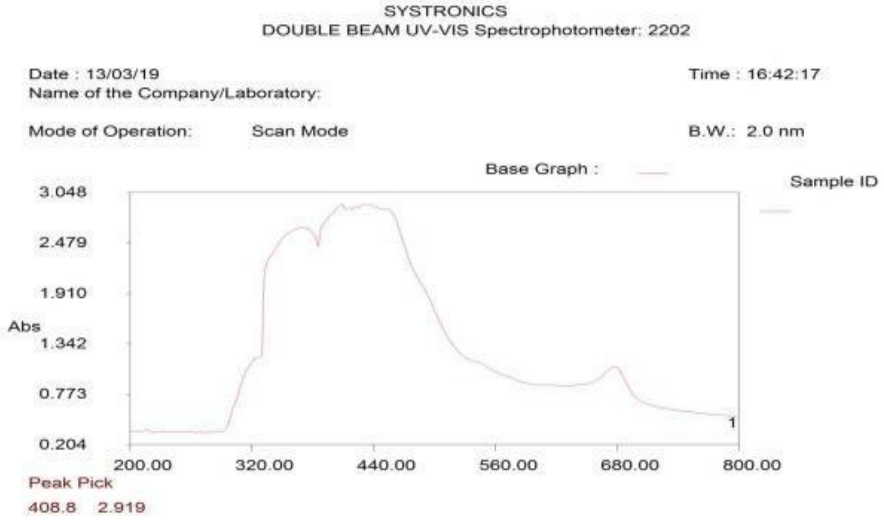

Fig. 4 UV-Vis spectra of *Lilium candidum* leaf extract silver nanoparticles

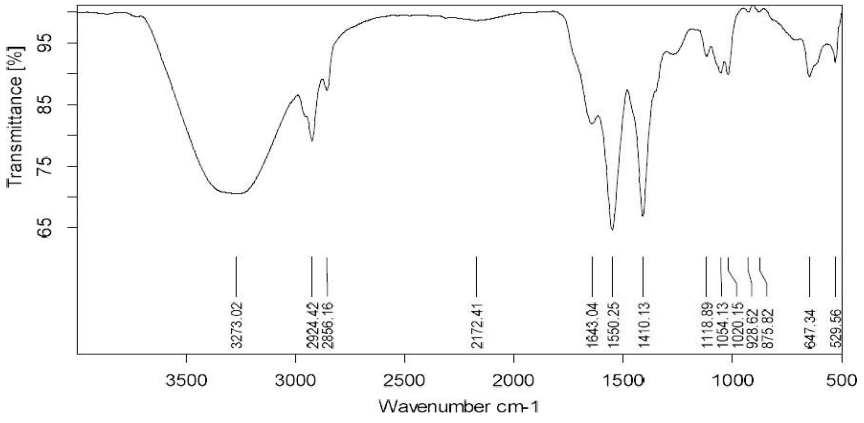

Fig. 5 FTIR of stem extract of *Lilium candidum*

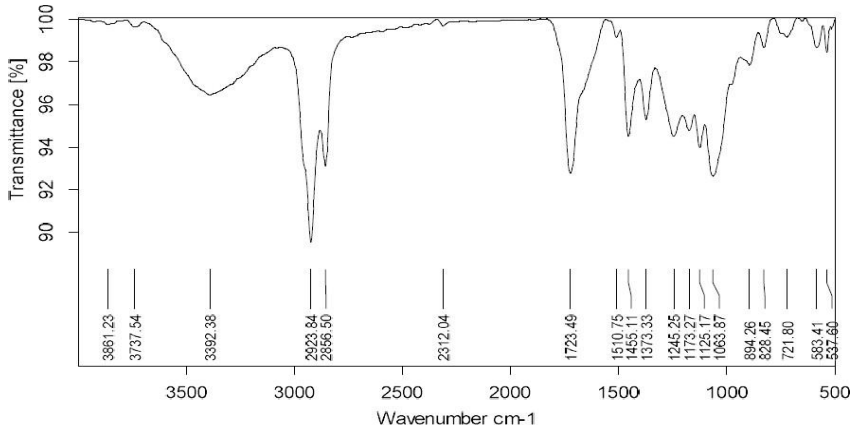

Fig. 6 FTIR of synthesized silver nanoparticles

Lilium candidum plant is also possessed well antimicrobial activity. To study the antibacterial property of plant extract, $AgNO_3$, silver nanoparticles (15ml), gram positive and gram negative bacteria were used and standard antibiotic ofloxacin were used in this study. Zone of inhibition against bacterial growth produced by silver nanoparticles was compared to standard antibiotic ofloxacin. From the table, it is concluded that synthesized silver nanoparticles exhibit inhibition zone nearly close to standard antibiotic values Table 6.

Table 6 Inhibition zone of extract, $AgNO_3$, silver nanoparticles and antibiotic against four bacteria

Bioactive agent	**Zone of inhibition (Diameter, cm)**			
	Actinomyces	***Streptococcus mutans***	***Proteous vulgaris***	***Pseudomonas aeruginosa***
Extract	2.2	1.5	2.1	2.2
$AgNO_3$	2.3	1.4	1.5	1.5
Silver nanoparticles	2.8	2.1	1.6	1.8
Ofloxacin	3.1	2.9	2.8	2.8

The data revealed that significant reduction in growth of test fungus *A. niger* and *C. Krusei* was observed with the extract, $AgNO_3$, synthesized silver nanoparticles and standard Amphotericin B shown in Table 7. Studies revealed that silver nanoparticles may kill fungal spores by destructing the membrane integrity or may interact with phosphorus and sulphur containing compounds and their interaction may cause damage to DNA and protein which lead to cell death. In the present study reduction in colony diameter on prepared plates is an indication of antifungal activity of silver nanoparticles. *C. krusei* was inhibited to higher extent comparative to *A. niger*.

Table 7 Inhibition zone of extract, $AgNO_3$, silver nanoparticles and antibiotic against fungi

Bioactive agent	**Zone of inhibition (diameter, cm)**	
	Aspergillus niger	***Candida krusei***
Extract	2.4	2.8
$AgNO_3$	2.3	2.6
Silver nanoparticles	2.6	2.8
Amphotericin B	2.6	3.0

4. CONCLUSION

The present investigation involved the screening and evaluation of *Lilium candidum* leaf crude extracts for the phytochemicals, antimicrobial activity and antioxidant activity. Green synthesis of silver nanoparticles using aqueous leaf

extract, characterization and antimicrobial activity of synthesized nanoparticles were also carried out in the study. Outcome of all the experiments carried out suggests the existence of most of the phytochemicals in the leaves and are having some important biological activities. Further work is needed to isolate, purify and identify the exact active principle which is the cause for the biological activities. The Green synthesis is a simple, low cost and ecofriendly approach without any huge inputs in terms of energy. This is the first report of green synthesis of silver nanoparticles for this plant. Being exhibiting greater antimicrobial activity, phytochemical based nanoparticles may stand as a potential remedy in developing drugs against antibiotic resistant bacteria.

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