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BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES BY USING CAJANUS *CAJAN* FLOWER EXTRACTS AND ITS ANTI-MICROBIAL ACTIVITIES

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ABSTRACT

Green synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers. In this concern, Indian flora has yet to divulge innumerable sources of cost –effective non-hazardous reducing and stabilizing compounds utilized in preparing AgNPs. This study investigates an efficient and sustainable route of AgNP preparation from 1mM aqueous $AgNO_3$ using flower extracts of Cajanus cajan Leguminosea family well adored for their wide availability and medicinal property. The AgNPs were characterization by UV-visible (Vis) spectrophotometer, Scanning electron microscopy (SEM). Fourier transform infrared spectrometer (FT-IR) analysis was carried out to determine the nature of the capping agents in each of these flower extracts. AgNPs obtained showed significantly higher antimicrobial activities against Staphylococcus aureus, and E.coli in comparison to both $AgNO_3$ and raw plant extracts. In totality, the AgNPs prepared are safe to be discharged in the environment and possibility utilized in processes of pollution remediation, may also be efficiently utilized in agricultural research to obtain better health of crop plants as shown by our study.

KEY WORDS

Green synthesis, AgNPs, Silver nanoparticles, UV, FTIR, XRD, SEM, antimicrobial property, etc.,

INTRODUCTION

Nanoparticles synthesis is usually carried out by various physical and chemical methods like laser ablation, pyrolysis, lithography, chemical vapour deposition, sol–gel techniques and electrodeposition, which are very expensive and hazardous. Although many routes are available for the synthesis of Nanoparticles, there is an increasing need to develop high-yield, low cost, non-toxic and environmentally friendly procedures. Therefore, scientists are looking forward for greener methods [1,2]. Green nanotechnology is an area of interest having significant focus in present scenario with important objective of facilitating the manufacture of nanotechnology based eco-friendly products. The development of efficient green chemistry methods for synthesis of metal Nanoparticles has become a major focus of researchers [3]. Among heavy metal Nanoparticles, silver Nanoparticles have received major

attention due to unique and tunable surface plasmon resonance (SPR) [4]. Synthesis of silver Nanoparticles with different size range and their self-assembly is considered important due to their potential applications in medicine [5]. In recent years, synthesis of AgNPs has been reported using several plant extracts particularly Prosopis juliflora [6]; Malva parviflora [7]; Hibiscus cannabinus [8]; Ocimum sactum [9]; Sesbania grandiflora [10]; Acalypha indica [11]; Alternathera sessilis [12]; Catharanthus roseus [13]; Ixora coccinea [14] and Mulberry [15].

Silver nanoparticles (AgNPs) were successfully obtained from bioreduction of silver nitrate solutions using Acalypha indica extracts. Owing to varying properties of these three-plant species, AgNPs obtained from the smallest being yield using Acalypha indica extracts. AgNPs have been appropriately characterized using UV-Vis spectroscopy, FTIR, XRD and TEM analysis. FTIR analysis revealed the efficient capping and stabilization properties of these AgNPs. The average size of synthesized silver nanoparticles using leaf extracts of Acalypha indica by XRD and TEM analysis was Similar and found to be 16.86 nm and 16.6 nm respectively. The leaf extract of Acalpha indica has a good anti-inflammatory activity. Hence, due to their benign, stable nature and anti-inflammatory property, these AgNPs may be well utilized in industrial and remedial purposes. However, plant uptake and utilization of AgNPs requires more detailed research on many issues like uptake potential of various species, process of uptake and translocation and the activities of the AgNPs at the cellular and molecular levels [16].

Synthesis of silver nanoparticles, reducing Ag+ ions present in the aqueous solution of silver nitrate by the help of onion extract. Through elaborate screening process involving number of plants, we observed that onion (Allium cepa) was potential candidate for synthesis of silver nanoparticles. We also study the antibacterial property of silver nanoparticles toward E.coli and Salmonella typhimurium. Although, several previous reporters have studied the antibacterial activity of chemically synthesized silver nanoparticles [17-19].

In the present study, we first report the reduction of silver ions using aqueous Cajanus cajan flower extract under microwave irradiation for facile and fast phytosynthesis of silver nanoparticles (AgNPs). To the best of our knowledge, no reports pertaining to a Cajanus *cajan* flower extract are yet available. The anti-microbial property of silver nanoparticles was also investigated.

MATERIALS AND METHODS

Preparation of flower extract

The fresh and young flower samples (Cajanus *cajan)* was collected and washed thoroughly with sterile double distilled water (DDW). Twenty gram of sterilized flower samples were taken and cut into small pieces. Finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile DDW After that, the mixture was boiled for 5 minutes and then filtered. The extract was stored in 4^0C.

Synthesis of silver nanoparticles

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. 100 ml flower extract was added to 100 ml of 0.1N $AgNO_3$ aqueous solution in conical flask of 250 ml content at room temperature. The flask was thereafter put into shaker (100 rpm) at 500 C and reaction was carried out for a period of 12 hrs. Then the mixture is kept in microwave oven for exposure of heat. The mixture was completely dried after a period of 20 minutes and hence Nanoparticles in form of powders were obtained.

UV visible spectroscopy analysis

The colour change in reaction mixture (metal ion solution + Cajanus *cajan* flower extract) was recorded through visual observation. The bio reduction of silver ions in aqueous solution was monitored by periodic sampling of solid and subsequently measuring UV visible spectra of the solid sample. UV-visible spectra of sample were monitored as a function of time of reaction on the UV-visible spectroscopy and the investigation was carried out using PERKIN ELMER (Lambda 35 model) spectrometer in the range of 190 nm to 1100 nm.

FT-IR Measurement

The Fourier transform infrared (FTIR) investigation is carried out using PERKIN ELMER (Spectrum RXI) spectrometer in the range of 400 cm^{-1} to 4000 cm^{-1}. The functional groups were identified using the peak assignments.

XRD Measurement

The sample was drop- coated onto Nickel plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample was prepared. The particle size and nature of the silver nanoparticle was determined using X-ray

diffraction (XRD). This was carried out using Rigaku miniflex-3 model with 30kv, 30mA with Cu kα radians at 2θ angle.

SEM Analysis

Sample is dispersed with acetone and exposed in ultrasonic for 5 minutes. Take a drop of a solution from the sample and drop it on the grid, leave until it dries. After drying the sample is inserted into SEM instruments using model is Tecnai T20 Making in FEI, Netherlands operating at 200 KeV Tungsten Filament.

Antibacterial activity

Microorganisms and culture media

Bacterial cultures such as*, Staphylococcus aureus*, *E.coli,* were obtained from Eumic analytical Lab and Research Institute, Tiruchirappalli. Bacterial strains were maintained on Nutrient agar slants (Hi media) at 4˚C.

Inoculum preparation

Bacterial cultures were sub-cultured in liquid medium (Nutrient broth) at 37˚C for 8h and further used for the test (10^5-10^6 CFU/ml). These suspensions were prepared immediately before the test was carried out.

Preparation of culture media

Nutrient agar medium

Nutrient agar medium is one of the most commonly used medium for several routine bacteriological purposes:

Ingredients	:	Grams/Litre
Peptone	:	5gm
Beef extract	:	3gm
Agar	:	15gm
Sodium chloride	:	5gm
Yeast extract	:	1.5gm
p^H	:	7.0

After adding all the ingredients into the distilled water, it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 Ib psi pressure (121˚C) for 15 minutes.

Nutrient broth

The nutrient broth was prepared by the same composition without agar. At the adding all the ingredients into the distilled water it is boiled to dissolve the medium completely and sterilized by autoclaving at 15 ib psi pressure (121˚C) for 15 minutes.

Preparation of plant material

Flowers, of the plant materials taken for this study were shade dried individually at room temperature and then powdered by using electric, blender. About 10gm of fresh plant materials (flower) were extracted with 100ml of distilled water 91:10. They were kept for seven days at room temperature (31˚C) for complete extraction. After seven days. The extracts were filtered through what man no.1 filter paper. This extract was collected in both and kept in refrigerator.

Continuous hot extraction using soxhlet apparatus

When concentrated preparations are manufactures, there is first extraction followed by evaporation. In the continuous both the operations i.e., extraction and evaporation are combined in the apparatus used for this purpose. To execute continuous not extraction a soxhlet apparatus is used soxhlet continuous extractor. The apparatus is used for the extraction on coarse drug powder placed in a thimble made of filter paper is inserted into the wide tube of the extractor. The solvent which is taken is taken in the flask is heated, the vapors arise from the solvent get in to the condenser through a side tube and the liquid condensed from the vapor's drips into the thimble. The solvent liquid level slowly rises and during this period the dried flower materials gets extracted of its soluble constituents. When the level of the liquid reaches the top of the siphon it gets siphoned into flask. The suction effect of the siphoning assists permeation of the solvent through the drug.

Again, a portion of the solvent from the solution vaporized leaving the constituents in the flask itself and the process mentioned above is repeated. The same process is repeated again and again until all the solutes are extracted. This kind of continuous not percolation (soxhlation) is undertaken when the active constituents are not readily soluble in the cold and are thermo labile e.g., grinder Oleoresin is extracted with ethanol.

Assay of antimicrobial activity

Microbial inoculum preparation:

The nutrient broth were prepared, then identified bacterial colonies were inoculated into the broth culture were used for antimicrobial activity.

Kirby bauer agar well diffusion assay

The nutrient agar medium was prepared and sterilized by autoclaving at 121˚C 15 lbs pressure for 15 minutes then aseptically poured the medium into the sterile petriplates and allowed to solidify the Bacterial broth culture was swabbed on each Petri plates using a sterile bud. Then wells were made by well cutter. The organic solvent extracts of flower were added to each well aseptically.

This procedure was repeated for each Petri plates then the petri plates were incubated at 37˚C for 24 hrs. After

incubation the plates were observed for the zone of inhibition.

UV-Visible Spectroscopy Analysis

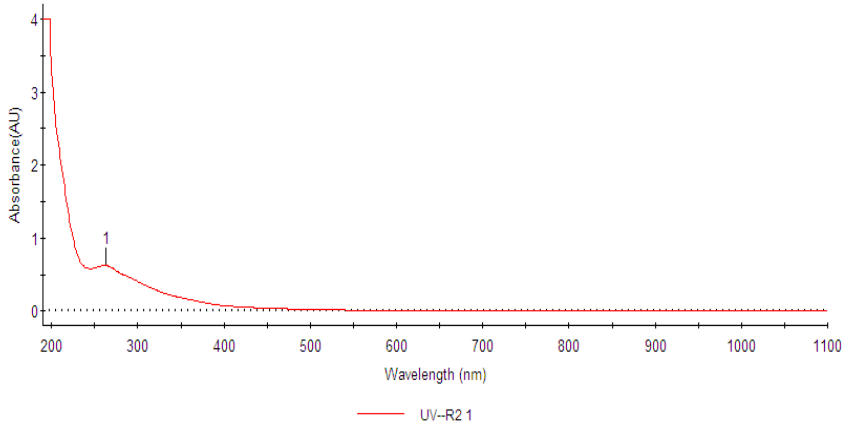

Fig.1 UV-Visible spectrum of synthesized silver nanoparticles using Cajanus *cajan* flower extract

UV-Visible spectroscopy analysis showed the absorbance band of silver nanoparticles synthesized using leguminosea flower extract at 262.50nm which conforms the presence of poly-unsaturated and aromatic compound (Isoguinoline) (Advanced strategies in food analysis, UV-VIS spectrometry International Journal of medicine and Pharmaceutical Research by Richart koplik.

FT-IR measurement

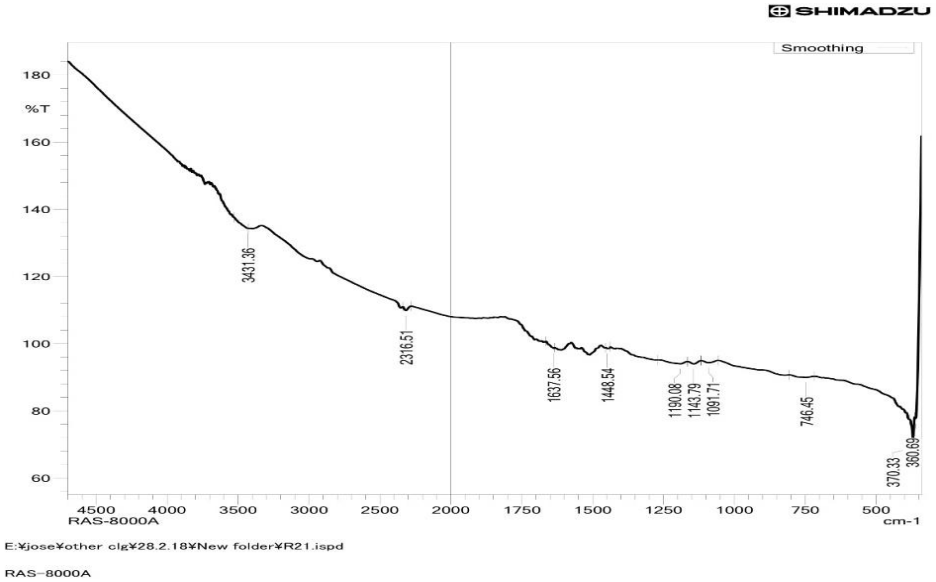

Fig.2 FT-IR Spectrum of synthesized silver nanoparticles using Cajanus *cajan* flower extract

The leguminosea related functional groups were identified using the peak assignments. A strong peak at 3431.36 cm $^{-1}$ was assigned to the OH stretching in alcohol group, the strong peak at 2312.65 cm^{-1} was assigned to O=C=O stretching Carbon-dioxide group, strong peak at 1637.56cm^{-1} was assigned to C=O Stretching Amide group, a strong peak at 1448.57 cm^{-1} was assigned to C-H stretching alkane groups are a strong peak at 1190.08cm^{-1} was assigned to C-F stretching alkyl halide groups, a strong peak at 1143.79cm^{-1} was assigned to C-F Stretching alkyl halide group, a strong peak at 1091.71 cm^{-1} was assigned to C-F stretching alkyl halide group, a strong peak at 746.45cm^{-1} was assigned to C-H stretching alkene group .

XRD measurement

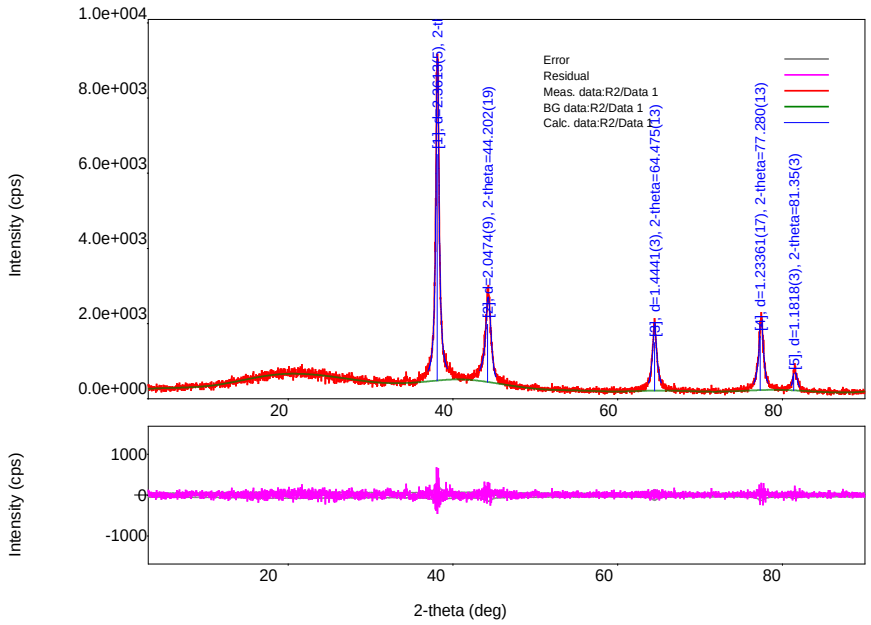

Fig.3 XRD Spectrum of synthesized silver nanoparticles using Cajanus *cajan* flower extract

Determination crystal

Line size

Gauss value

Particle size D =18.93261nm

Surface area S = 46.6942 m^2/g

SEM analysis

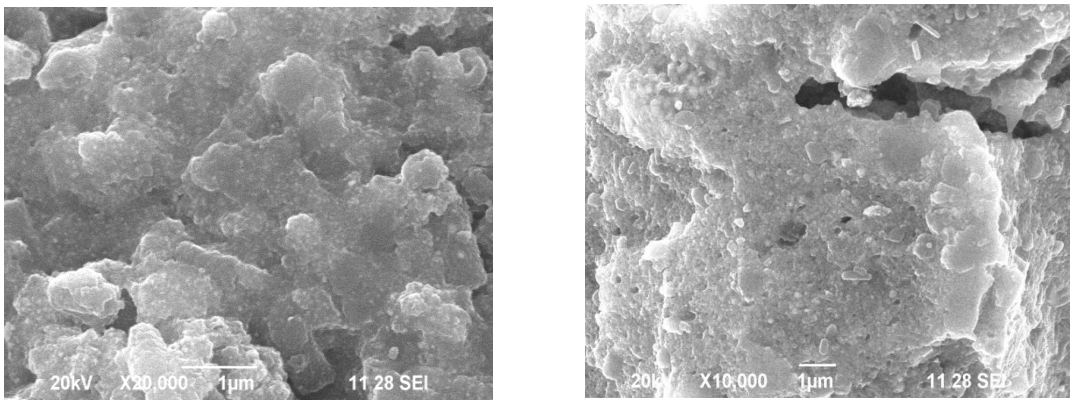

Fig.4 SEM image of synthesized silver nanoparticles using Cajanus *cajan* flower extract

SEM analysis shows uniformly distributed silver nanoparticles on the surface of the cells. SEM analysis reveals individual spherical polydispersed AgNPs as well as number of aggregates, which were nanorod in shape. The size of the silver nanoparticles was found to be 5-30nm, with an average size 19.2nm. The larger silver particles may be due to the aggregation of the smaller ones.

Anti-microbial activity

In the present, isolated ethyl acetate fraction of leguminosea maxima flowers exhibited significant anti-microbial activity when compared with standard drug. It is evident from the data presented in table 1 that the sample possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 25mg/ml as 12mm, and 10mm, for 50mg/ml showing 14mm and 12mm 75mg/ml showed 16mm

and 14mm for 100mg/ml as 23mm,and 20mm,against staphylococcus aureus ,E.coli respectively when compared with standard drug Gentamicin showing 23mm, and 20mm zone of inhibition respectively . Then it is evident from the data presented in table that the sample possesses antibacterial activity. The above result shows that the activity of the compound isolated from leguminosea maxima flower shows significant antibacterial activities.

Table.1 Anti-bacterial activity of the compound isolated from Cajanus *cajan* flower in different strains

SAMBLE	Extract 100 µl added and Zone of inhibition (mm/ml)				
	25 µl	50 µl	75 µl	100 µl	Control
Staphylococcus aureus	12	14	16	18	23
E.coli	10	12	14	16	20

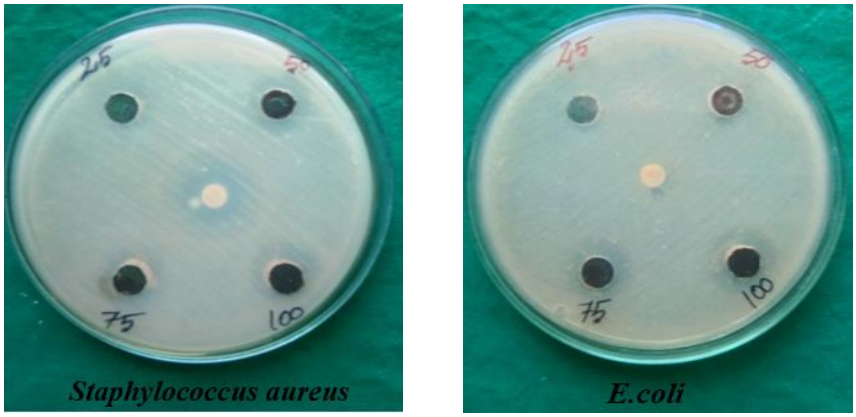

Fig.5 Graphical representation of anti- bacterial activity of the compound isolated from Cajanus *cajan* flowers (standard: Gentamicin, concentration 1mg/ml)

CONCLUSION

Based on the result of the above study on the Cajanus *cajan* we conclude that the compound isolated from Cajanus *cajan* flowers shows superior anti- bacterial activity against the following microorganisms such as Staphylococcus aureus. Also, it justifies the claimed uses of flower parts of the Cajanus *cajan* in the traditional system of medicine to treat various infections disease caused by the microbes. Antimicrobial activities are aggravated by increasing the quantity of this compound, which can be used as an alternative for antibiotics. Therefore, it is necessary to characterization their active compounds and should be investigated for better understanding of its safety, efficacy and properties.

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