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Biogenic Nanosilver Mediated by Coir, Medicinal Plant Extracts and their Antimicrobial Validation

Geethanjali Kanagaraju*, Sumy Sebastian and Anita Das Ravindranath

Abstract

Knowing the magnitude of therapeutic plants such as *Micrococca mercurialis* (MM), *Abutilon palmeri* (AP) and *Callistemon citrinus* (CC), we also planned to extend our research work on *Cocusnucifera* fibers (CF) and dust (CD) as it has copious medicinal properties. The current study deals with the green synthesis of silver nanoparticles (AgNPs) from fresh aqueous extracts and $AgNO_3$ solution. The biogenic conversion of silver ion to silver is relatively expeditious at two different time intervals and pH. The isolated silver nanoparticles (AgNPs) from the bio extracts were identified initially by scrutinizing the colour variations. The biosynthesized AgNPs were characterized by UV, XRD, Laser Diffraction Particle size Analyser, fluorescence spectroscopy, SEM and TEM. UV absorbance at 435-460nm for silver nanoparticles was observed for the above extracts. The XRD pattern of all extracts showed the distinctive Bragg peaks of face centre cubic (fcc) crystalline system available in nature. SEM and TEM analysis of the silver nanoparticles indicated that the particle size was in the range of 2-100nm with polygonal and spherical shapes. The biosynthesized AgNPs were evaluated for antimicrobial activities. The CFAgNPs showed an efficient antibacterial activity at lower concentration (25mg/ml) against *Pseudomonas demolytica* followed by *Staphylococcus aureus* and *Escherichia coli*. Similarly all plant extracts have better activity against fungal strains. It is concluded that the biogenic blend of AgNPs is simple, extremely rapid, beneficial, eco-friendly and more stable without any toxic effects. Of these, CFAgNps may be used for the preparation of antibacterial groupings against *Pseudomonas demolytica* and *Staphylococcus aureus*.

Key words: Green synthesis, Silver nanoparticles, Plant extracts, Antimicrobial activity

Introduction

In the current scenario, Nano-biotechnology find extensive applications of nano-medicine, an emerging new pasture which is an upshot of blending of nanotechnology and medicine [1**]**. Nano particles bound to drugs are easily suspended in liquids and penetrate deep in organs and tissues than the conventional form of drugs [2]. Precise nanoparticle engineered drugs have capitulated longer circulation half-life, advanced accessibility and lesser toxicity [3]. The therapeutic importance of various nanometals such as Ti, Zn, Cu, Mg Au and Ag are cited in ancient Indian Ayurvedic medicinal book named "Charak Samhita" [4]. Among the metals, silver nanoparticles (AgNPs) play a profound task in the field of chemical, biology, medicine and environmental remediation due to their conspicuous physiochemical properties. Silver products are known to have inimitable properties in area of catalysis [5] chemical sensing, biosensing [6] drug delivery [7] photonics, electronics [8] cosmetics [9] water treatment [10] paints [11] agriculture [12] and a broad spectrum of antibacterial activity [13-15] antifungal [16] anti-inflammatory, antiviral, arthroplasty, antiangiogenesis, and antiplatelet activity [17].

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AgNps have been synthesized by physio-chemical procedures such as chemical reduction, micro emulsion, electro chemical method, [18] photochemical reduction [19] gamma ray radiation [20] laser ablation [21] autoclave [22] and microwave [23]. Nevertheless, these processes are noxious and costly. Biological systems like microorganisms and floras have been reported to synthesize various metal/ metal oxide nanoparticles. Among the synthesis procedures of production of nanoparticles, the plant-mediated technique is gaining essence due to its one step, simplicity, eco-friendliness, cost-effective and comparatively more stability. The biogenic nanoparticles revealed entirely novel or superior properties based on explicit uniqueness such as size, distribution and morphology. The bio-active molecule present in aqueous extract of the source has the ability to be absorbed on the surface of nanoparticles during particle formation procedures and eventually leads to the occurrence of ensuing different surface property throughout their application. Therefore, evaluating the different plant sources which have different constituents as a mediate for nanoparticles production might be remarkable. Moreover, utilizing plant extracts also shrink the cost of microbes, isolation and culture media enhancing the charge competitive feasibility over nanoparticles synthesis by microorganisms.

This paper describes the synthesis and characterization of AgNPs, a biosynthetic approach of using plant extracts as a reducing agent in addition to surface stabilizing agents for the synthesis of Ag-NPs. The application of these plant extracts for pharmaceutical purposes and isolation of resultant metabolites has been reported by various authors, but synthesis of Ag-NPs are scanty. Hence the current study, was undertaken to explore and extend the comparative study of biogenic synthesis of Ag-NPs by using medicinal plants with *C. nucifera* fibre/dust extracts and characterize their NPs using UV, FT-IR, XRD, fluorescent spectroscopy, SEM and TEM. Furthermore, we have assessed their medicinal potential as the antimicrobial mediator against, *Pseudomonas demolytica, Escherechia coli, Staphylococcus aureus, Aspergillus niger, Candida albicans* and *Cryptococcus neoformans.*

Material and Methods

Material collection

Three Indian medicinal plants such as *Micrococca mercurialis* (MM) *Abutilon palmeri* (AP) and *Callistemon citrinus* (CC) were collected from Coimbatore, Tamil Nadu, and *Cocosnucifera* fibres (CF) and dust (CD) were collected from CCRI, Kalavoor, Kerala, India, on the basis of cost effectiveness, ease of availability and medicinal property.

Preparation of plant extracts

Fresh and healthy leaves were rinsed thoroughly first with tap water followed by distilled water to remove all unwanted visible particles, and free of disease cut into small pieces and dried at room temperature. About 20 g of these finely incised leaves of each plant type were weighed separately and transferred into 250 mL Erlenmeyer flask containing 75 mL distilled water and boiled for about 1hr. The extracts were then filtered thrice through Whatman No. 42 filter paper to remove particulate material and to get clear solutions which were then refrigerated at 4°C. Throughout the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination.

Preparation of *Cocusnucifera* fibres and dust extracts.

Fresh *C. nucifera* fibres and dust were washed thoroughly 2-3 times with tap water and with distilled water. 50 g of fresh fibers was incised into small pieces and added to 100mL of distilled water and stirred at 70°C for 2 hrs. After boiling the extract was cooled and filtered with Whatman paper number 42. Filtrate was collected. Similarly 20g of *C. nucifera* dust was washed and added 75ml of distilled water and stirred at 70°C for 2 hrs .The extract was cooled down and filtered with Whatman filter paper 42 and both the extracts were stored at 4°C.The extracts may act as reducing as well as stabilizing mediators in the synthesis of AgNPs.

Green Synthesis of Silver Nanoparticles

The concentration of the plant extract, metallic salt solution, and temperature, pH and contact time are important factors which influences the rate of production of the nanoparticles, their quantity and other characteristics. About 50 mL (0.01N) aqueous solution of silver nitrate was prepared in Erlenmeyer flask. Sodium hydroxide was used to keep up higher pH nearly12. Then 5mL silver nitrate solution was added drop wise separately to 20mL of each aqueous extract solution kept in separate beakers. The solution was then mixed thoroughly by a magnetic stirrer. Initially at 50°C there were no colour changes when the reaction system was maintained at pH2. While the reaction temperature and pH increased to 70°Cat pH 12 precipitations occurred. The reaction time sustained for 1 hr until solution colour changed to faint to dark brown exponentially indicating the formation of silver nanoparticles. After 60 minutes there was no significant change in colour which is evidence for the completion of reduction process due to Surface Plasmon Resonance of silver nanoparticles [38]. The collected silver NP's were allowed to dry in a beaker. The bioreduction of silver ions was screened by periodic sampling by the UV spectrophotometer.

Characterization of synthesized silver nanoparticles

The UV–Vis spectroscopy measurements were recorded on a JASCO dual beam spectrophotometer (Model SHIMADZU UV-1650) operated at a resolution of 2 nm. Photoluminescence (PL) spectra were recorded using Perkin Elmer LS 55 fluorescence spectrometer. Fourier Transform Infrared Spectrometer spectra were recorded under identical conditions in the 400-4000 cm-1 region using Fourier Transform Infrared Spectrometer (spectrum RX-I, FT-IR system, Perkin eliner Model). Photoluminescence spectra were recorded in JOBIN YVON FLUROLOG-3-11. The emission scan was carried out in the range of 300–800 nm resolution 0.2 nm. The data were analyzed using the Software DATA MAX/ GRAMS/31. The phyto reduced silver colloidal solution was drop-coated onto a glass plate; and the XRD measurements were carried out using a X-ray powder diffract meter (Bruker AXS D8 Advance: Anton Paar, TTK 450), on the instrument operating at a voltage of 200 kV and Max. Usable angular range: 3° to 135°. Laser Diffraction Particle size Analyser LS13320 was used to establish the average size of synthesized silver nanoparticles. Morphological characterization of the samples was carried out using TEM-JEOL/JEM2100 instrument at an accelerating voltage of 200 kV and SEM-JSM-6380, (JSM 63801LV) Accelerating Voltage of 30kV. A pinch of dried sample was coated on a carbon tape. It was again coated with gold in an auto fine coater and then the material was subjected to analyze the morphological changes.

Antibacterial Assay

The antibacterial activity of synthesized AgNPs, AgNO3, control Gentamycin and bio extracts were tested against bacterial strains like *Staphylococcus aureus*, *Escherechia coli* and *Pseudomonas desmolytica.* Different dilutions of biosynthesized AgNPs varying from 25 to 100 mg/ml were prepared with twofold symmetry. Fifteen petri plates were taken and 25 ml of nutrient agar media was poured into these petri plates and inoculated with each bacterial pathogen. Different dilutions of each bacterial strain was prepared and standardized to match the McFarland (turbidity) standard, in which the bacterial density was estimated to be 1.5×10^8 CFU/ml. Then the plates were spun slowly for uniform distribution of the inoculum and left at room temperature for 7-10 min with the lid closed. When the agar gets solidified, three wells (5 mm diameter) were made in each Petri plate and each well was filled with different dilutions of AgNPs (100 mg/ml, 50 mg/ml, 25 mg/ml solution) of each source extracts. All the plates were incubated at 35°C for 24 hrs. After the pre determined time, the zone of inhibition formed at the minimum dilution for each pathogen was traced. Gentamycin was used as positive control.

Antifungal activity

The antifungal activity was tested against *Candida albicans* and *A*spergillus *niger, Cryptococcus neoformans*. The well diffusion

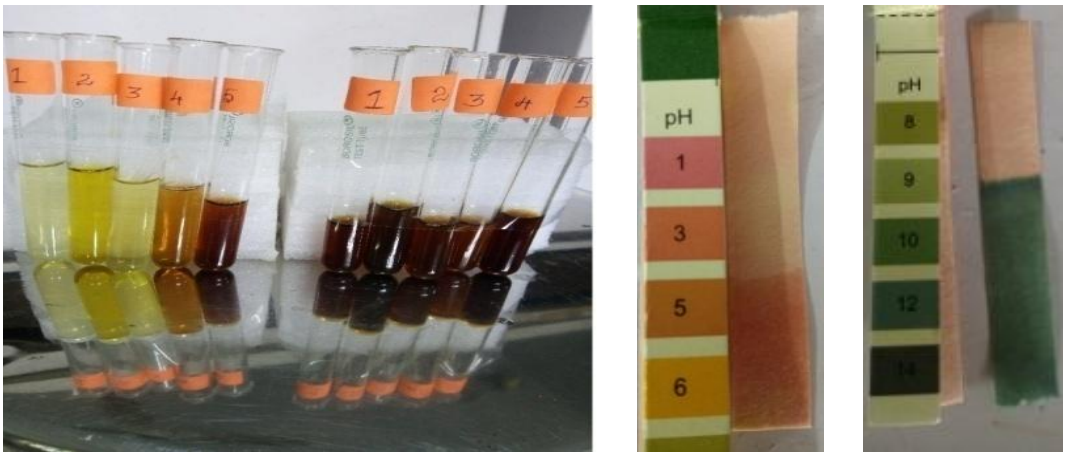


Figure 1

test was performed using Muller Hinton Agar no. 2. The inoculum was prepared in sterile Nutrient Broth (Peptone 10 gm/L, Beef extract 10 gm/L, Sodium chloride 5 gm/L, pH 7.2) and the tube was incubated at 37˚C until the turbidity was achieved up to the 0.5 McFarland standard. The Mueller-Hinton agar no. 2 plate was inoculated with 2 ml of inoculum by streaking the swab over plate. Then, agar was punched with help of sterile borer to create 5 mm well. 100mg/ml, 50mg/ml and 25mg/ml of different concentrations of AgNp extracts were added in respective wells. Silver nitrate (1 mM) was used as a control. Plates were incubated at 37◦C for 48 hrs. The diameter of inhibition zone was measured. To assess the effect of AgNPs obtained and the standard antibiotic Fluconazole was used.

Results and Discussion

In order to confirm the formation of AgNPs in the extracts UV-Vis spectra was recorded at 70˚C, initially the colour of the reaction mixture changes from pale brown within 40 min and colloidal brown after 60 min confirming the completion of the reaction shown in **Fig. 1.** Absorption spectra of all AgNPs formed in the reaction media has absorption maxima in-between the range of 435 and 460 nm for reaction conducted at 70˚C, with minimum time interval at 40 min & 60 min respectively. As the reaction temperature reaches 50˚C there was no significant colour change, as the temperature arises to 70˚C both synthesis rate and final conversion to silver nanoparticles increased. The time essential for the final conversion was found to be absolute at almost 60 min at 70˚C due to absorptionof metallic Ag nanoparticles for the unique process of surface plasm on resonance of AgNPs. This may due to the communal result of vibration of electrons of the silver nanoparticles in resonance with the light waves. The UV-Vis spectra recorded, implied that most rapid bioreduction was achieved in plant extracts followed by coir fibre and coir dust extracts as the reducing agent. The peak broadening indicates the pattern of polydispersed large nanoparticles due to slow reduction rates [39]. It was noted that the absorption gradually raises in intensity as a function of time and temperature of reaction, indicating an increase in the number of Ag-NPs formed in the solution [40]. The extracts CCAg, APAg, MMAg, CFAg and CDAg typically show absorption band at 425, 430, 435, 450 nm and 460nm respectively shown in **Fig. 2**. The increase in intensity of the peak designates maximum number of nanoparticles formed as a result of reduction of silver ions presented in the extracts. The UV absorbance was also noted after 2 months the data showed no disparity. This confirms the degree of solidity of bio synthesised silver nano particles.

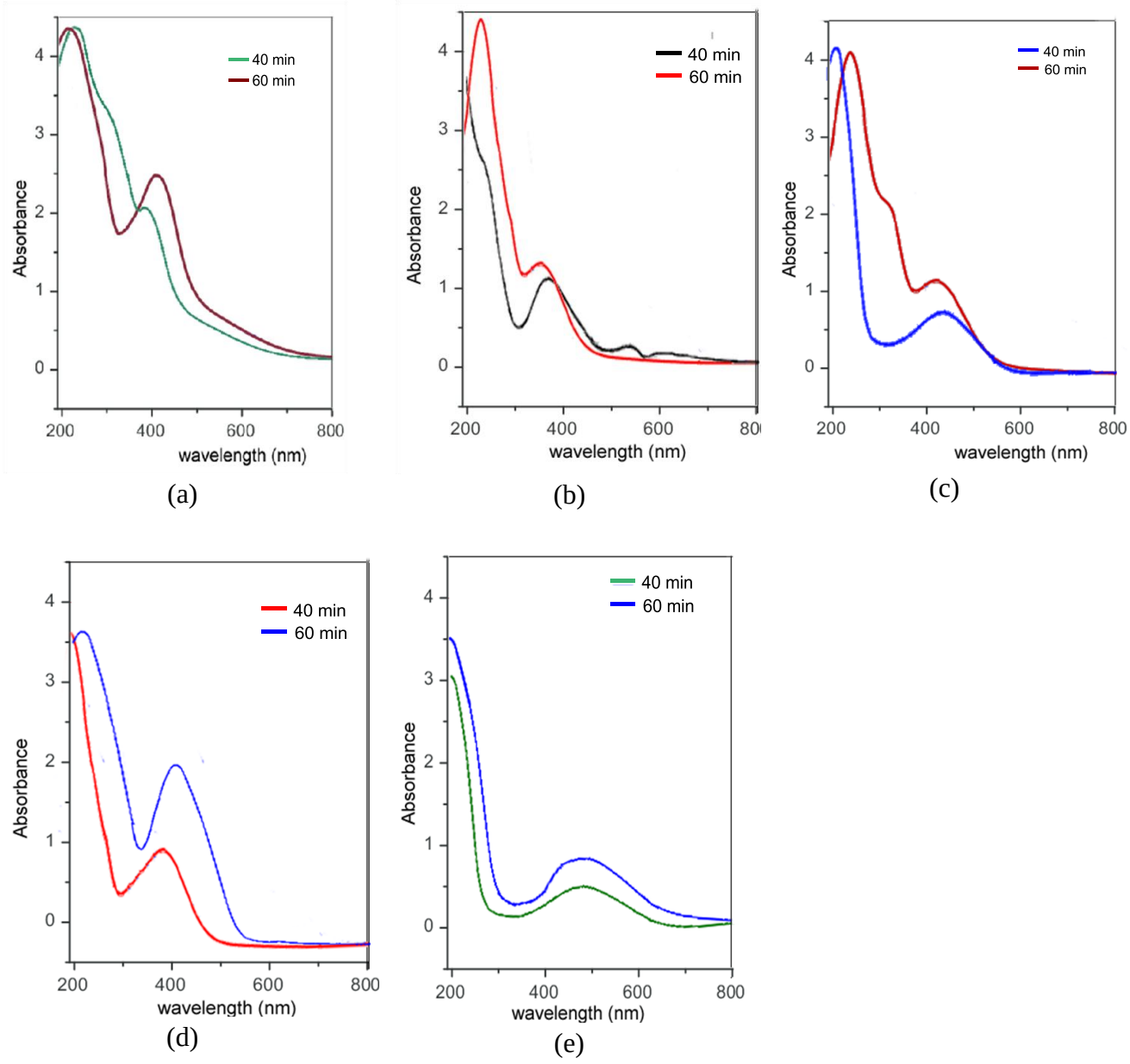


Figure 2

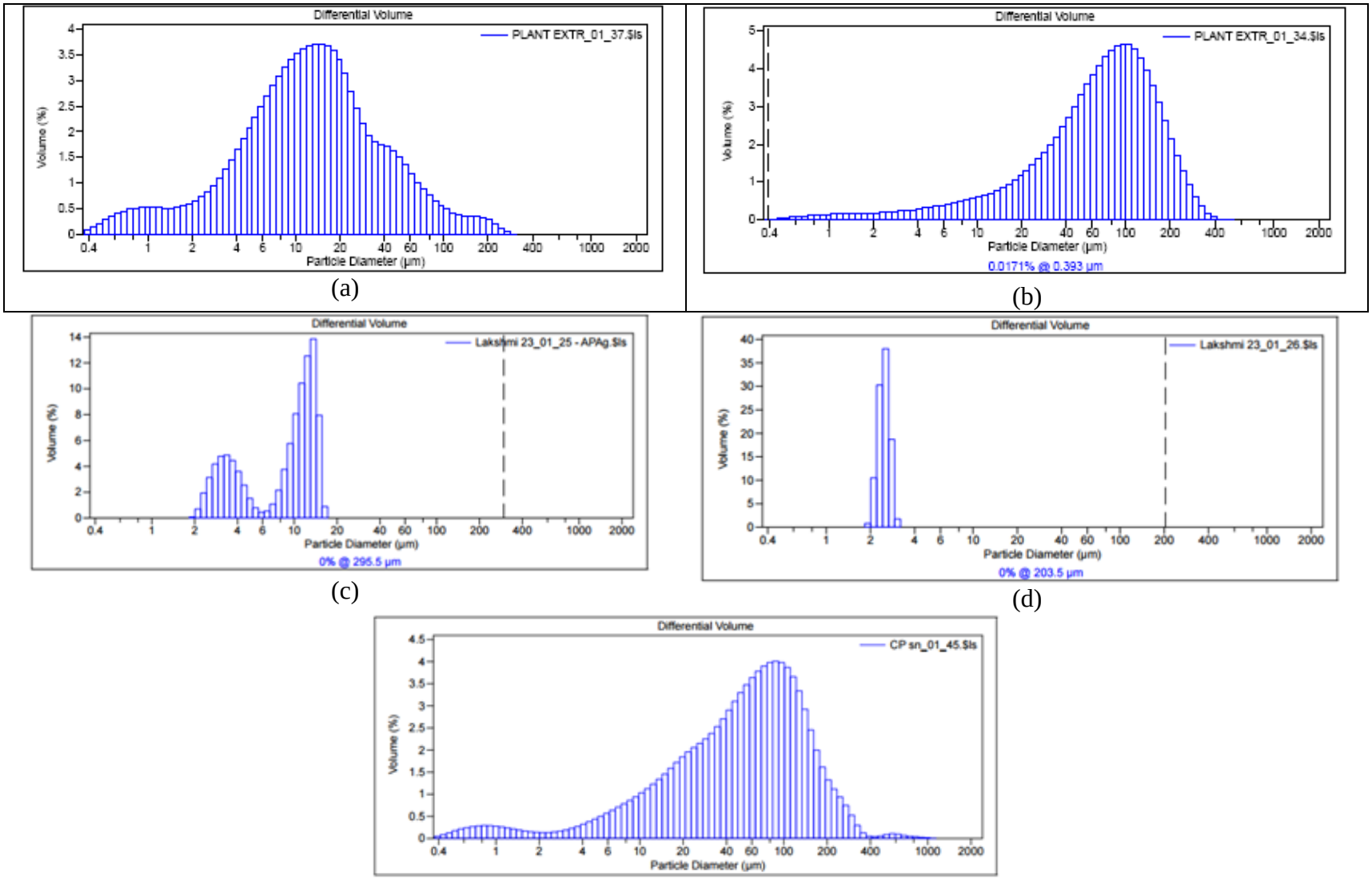
Effect of pH

The effect of pH of the reaction mixture on conversion and particle size with 20ml aqueous extracts was studied. The tint of the reaction medium and the effectiveness of the absorbance peaks were pH dependent. The reactions were monitored at pH 3 to 12. When the reaction commenced at pH3 neither colour change nor characteristic peak of AgNPs were noted. This may be due to the inactivity of bio molecules at lower pH. At pH 5-8 variation of colour changes occurred and the intensity of the colour rose to maximum at pH12. It is in agreement with literature reported by [41]. It may be evaluated that particle size and shapes are dependent on various conditions such as plant source, reaction temperature, composition of the mixtures, bio molecules and pH. As the reaction gets optimized at particular temperature, time and pH thereby conversion of most of the silver ions were consumed in the formation of nuclei, preventing the secondary reduction route on the surface of the preformed nuclei core. This may be due to the proteins containing amine groups partake in the reduction and controlling process during nanoparticle synthesis with change in its secondary structure after the reaction with silver ions [42]. Fig. 2 represents the results of the reaction obtained at two different time interval at 40 min and 60 min at pH3 and 12 respectively at 70°C.

The Laser Diffraction Particle size Analyser pattern of Ag nanoparticle suspensions synthesized using bioextracts were observed. Fig. 3 shows the size distribution histogram which indicates that the size of these silver nanoparticles varies between 20 and 40 nm and shows an asymmetric allocation. The shape of almost all plant mediated AgNPs were typically spherical with exception of *Micrococcus mercurialis* which yielded polydisperse particles both with spherical and flat plate like pentagonal hexagonal morphology. AgNPs synthesized from *Callistemon citrinus* leaves extract ranged at an average size of 25–35nm with few large size nanoparticles at 100nm. *Abutilon palmeri* mediated AgNPs ranged from 25 to 30nm whereas *Cocos nucifera* fibres (CFAg) and Dust (CDAg) yielded average diameter of 25-35nm and 30-35nm respectively at 70°Cof 2-50nm in size. High intensity allocation at lower range of particle size indicates that the most of the synthesized particles are in lower range of particle size. All the obtained data are in good agreement with XRD results of crystalline sizes.

Parameters for the crystalline size of the AgNPs determination Table 1 from X-ray line broadening by Debye–Scherrer formula given as $D = K\lambda/\beta \cos\theta$, where D is the average crystalline size (°A), *K* is a dimensionless shape factor, with a value close to unity. λ the X-ray wavelength used (nm), β the angular line width at half maximum intensity (radians) and θ the Braggs angle in degrees. On examining XRD pattern of silver nanoparticles of CC with prominent peaks at 2 theta 38.18°, 43.91°, 65.15°, 76.39°, represents (111), (200), (220), (311) and (222) attributed to Bragg's reflections of the FCC structure of silver with the variety of polyhedral and spherical shapes. X-ray pattern of AP silver nanoparticles are parallel with the FCC structure of the bulk silver with the peaks at 38.11º and 44.04º. These peaks correspond to (111), (200) planes, respectively. However unassigned peaks were also observed suggesting that the crystallization of bio-organic compounds occurs on the surface of the plant extract. Similarly for the aqueous extract of MMAgNPs the 2θ peaks at 38.06, 64.32 and 77.33 are indexed as (111) (220) and (311) planes respectively of FCC silver. With regard to XRD pattern of the CF derived AgNPs Fig. 4 shows three intense and weak peaks in the whole spectrum of 2θ values ranging from 20° to 80°. The 2θ *peak* values at 27.22, 31.84, 45.71, 66.98 and 76.33, which correspond to (200), (200), (200) (220) and (311) planes respectively for silver corresponds to FCC system. The intense peak of CDAgNPs appeared at 38.48°, 45.02°, 64.58° and 77.34°, which are indexed as FCC crystalline silver.

Silver nanoparticles are reported to exhibit visible photoluminescence arising from interband transitions. The luminescence spectra of CCAg, APAg, MMAg, CFAg and CDAg nano particles emitted at 470, 460, 455, 425, 440 nm respectively. In previous report it is stated that silver nanoparticles synthesized from olive


Differential Volume
PLANT EXTR_01_37.$ls
Volume (%)
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(a)
Differential Volume
PLANT EXTR_01_34.$ls
Volume (%)
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0.0171% @ 0.393 µm
(b)
Differential Volume
Lakshmi 23_01_25 - APAg.$ls
Volume (%)
Particle Diameter (µm)
0% @ 295.5 µm
(c)
Differential Volume
Lakshmi 23_01_26.$ls
Volume (%)
Particle Diameter (µm)
0% @ 203.5 µm
(d)
Differential Volume
CP sn_01_45.$ls
Volume (%)
Particle Diameter (µm)
(e)

Figure 3

Table 1

Samples	2θ	d-Spacing	Crystallite size (nm)	Lattice strain	hkl planes
CCAgNP	38.18	2.295	29.28	0.0038	1 1 1
	43.91	2.052	29.83	0.0032	2 0 0
	65.15	1.393	32.83	0.0020	2 2 0
	76.39	1.239	35.20	0.0017	3 1 1
APAgNP	38.11	2.301	29.27	0.0038	1 1 1
	44.04	2.002	29.85	0.0032	2 0 0
MMAgNP	38.06	2.324	29.27	0.0038	1 1 1
	64.32	1.467	32.68	0.0021	2 2 0
	77.33	1.246	35.43	0.0016	3 1 1
CFAgNP	45.71	1.987	30.03	0.0031	2 0 0
	66.98	1.425	33.17	0.0020	2 2 0
	76.33	1.247	35.19	0.0017	3 11
CDAgNP	38.48	2.098	29.31	0.0038	1 1 1
	45.02	2.010	29.95	0.0032	2 0 0
	64.58	1.423	32.73	0.0021	2 2 0
	77.34	1.225	35.43	0.0016	3 1 1

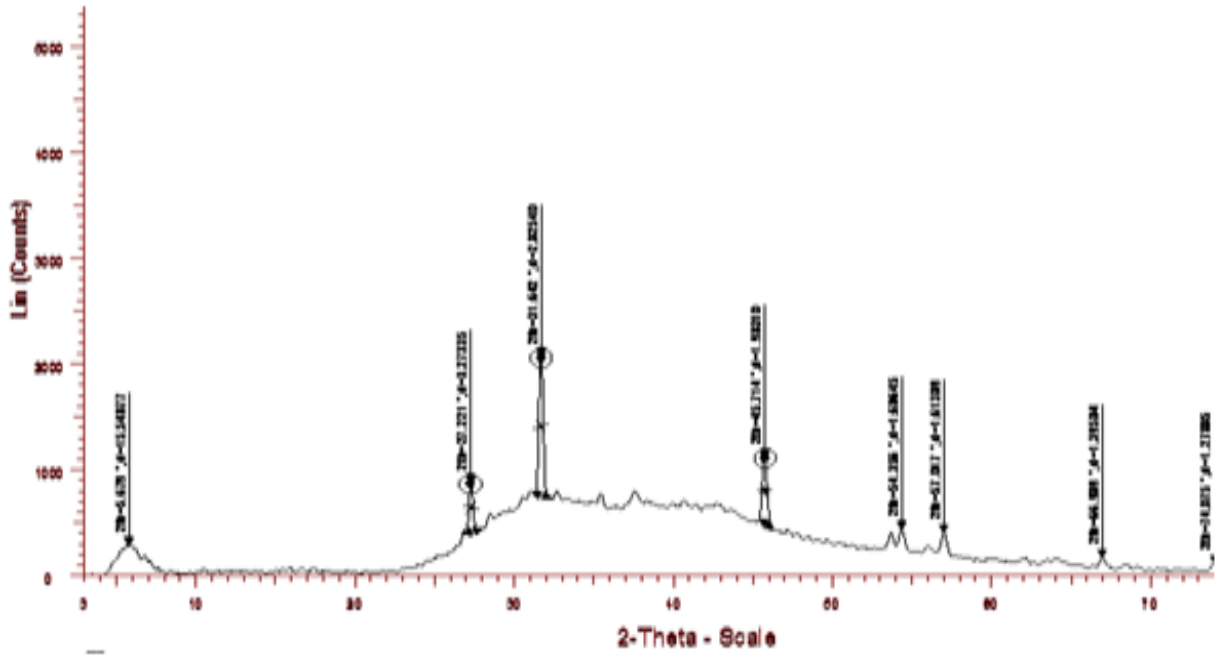


Figure 4

leaf extract reported the photo luminescent emission peak was observed at 425 nm. Generally fluorescence intensity varied directly with the absorption spectra and the position of fluorescence signal was found to be directly dependent to the nanoparticle size and surface functionalization [43].

SEM images of the silver nanoparticles are shown in Fig. 5. As the temperature increases more number of active sites are available for the reduction of silver ion and formation of large number of nanoparticles gives rise to intense peak thereby resulting in a more intense brown color.

Fig. 6 are TEM images, obtained with CF and 0.01NAgNO3 solution at 70° C. It is noted that the facets of AgNPs are between 20–30 nm in width, spherical shape of 2nm and few microns in length. Nevertheless the dimensions AgNPs of other plant extracts are not uniform. Along with these irregular structures the subsistence of numerous nanoparticles was also observed. Fig. 7 shows selected area electron diffraction pattern of the silver nanoparticles. The spots obtained reveals that silver particles are crystalline, face-centered cubic structure of elemental silver.

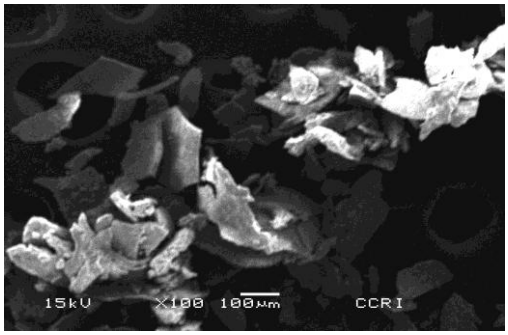
FT-IR measurements were carried out to categorize the potential biomolecules responsible for the reduction of the Ag+ ions and the ceiling of the biosynthesized silver nanoparticles. The band strength in different sections of the spectrum for the synthesized silver nanoparticles were analyzed and are shown in Fig. 8. The extracts CCAg, APAg, MMAg, CFAg and CDAg showed characteristic broad peak at 3257, 3397, 3149, 3158 and 3378.80 cm^{-1} respectively which corresponds to the stretching vibrations of phenolic and alcoholic O-H group. An intense sharp at 1383, 1383, 1383, 1383, 1372.88cm^{-1} respectively proved the presence of C-N group in the aqueous extracts. Similarly the peaks at 1629.35, 1620.45, 1606.86, 1600.26, 1609.91 cm^{-1} respectively corresponds to stretching/ bending vibrations indicating the complex formation of NH3+ between amide of C=O group proteins and silver NPs. A band at 1098.83, 1107.84, 1035.67, 1037.72 cm^{-1} respectively could be assigned to C-OH stretching of secondary alcohols excessively present in plants extracts. The bands at 825-600cm^{-1} could be attributed to C-H out of plane bending corresponds to aromatic poly phenols. The peaks at 2600, 2380, 2364, 2365, 2357 which are assigned to alkyne groups which are present in the phyto constituents of the extracts.

Antimicrobial activity of Silver nanoparticles

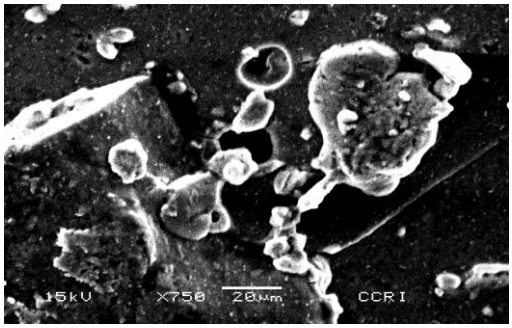
There are several literature reports proposing the mechanisms are the adhering capability of AgNPs to the microbial cell wall and cause cracks and pits ultimately to death of the cell. Other probabilities due to the catalytic oxidation of Ag^{+} with thiols lead to inactivate the 30S ribosomal subunit of protein the other co-enzymes and ion transport mechanisms. This probably leads to the malfunction of the cell by affecting the genomic DNA replication and thus killing the microbes [44].

It may also be considered that reactive H^{+} ions formed during the conversion of the keto form of the phenolic acid to unstable enol form, may be responsible for the formation of Ag^{0}. Silver ions are able to form complexes with polyols including phenolics, alkaloids, terpenoids glycosides, flavonoids and tannins. These bio molecules not only reduce ions to nano size and also play an imperative role in the capping of the nanoparticles shown in Scheme 1. The strength of the AgNPs can be endorsed to the formation of thin layer of silver complex electride on the aqueous surface of the reaction mixture. This complex salt possibly may convert the silver to nanoparticles.

It was also anticipated that Ag^{+} ion pierces the cell and intercalates between the nitrogenous bases like purine and pyrimidine by disrupting the hydrogen bonding between the two anti-parallel strands and disrupting the unique property of the DNA molecule [45] Bacterial cell lysis could be one of reason for its antibacterial property. The bio synthesized silver nanoparticles found to be accumulated in the bacterial membrane which results in the increase in permeability and death of the cells. The exact nature of the synthesis of NPs in these biological sources are unknown.



(a)



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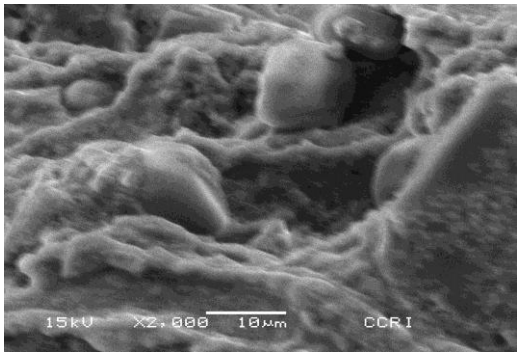

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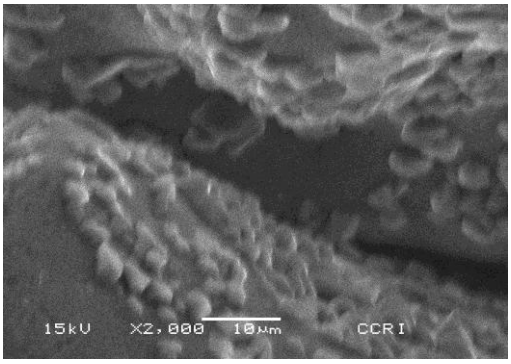

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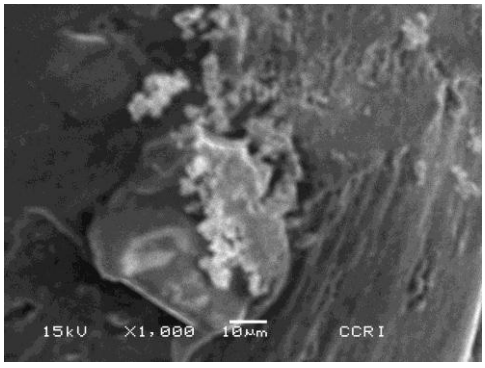

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Figure 5

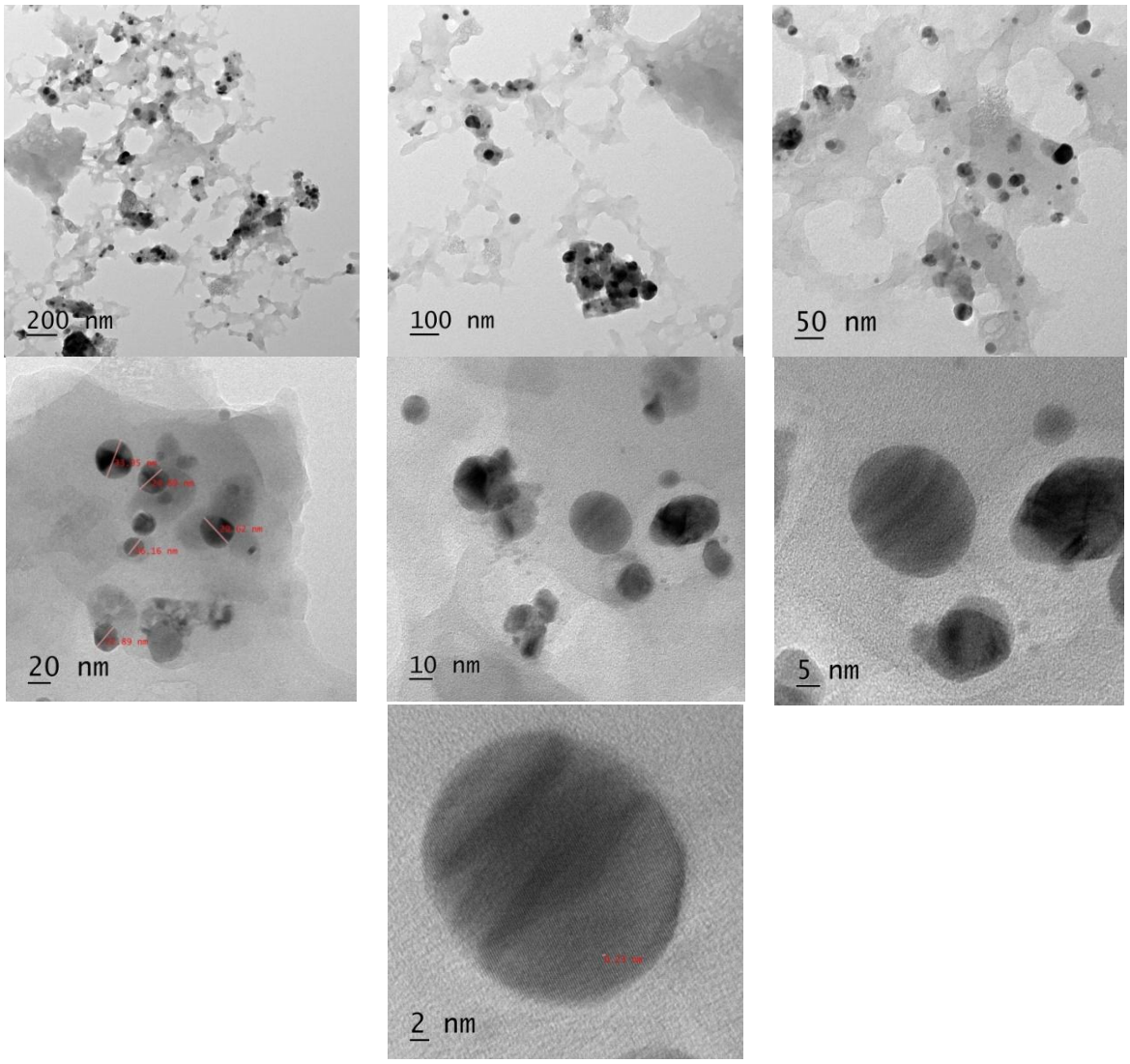


Figure 6

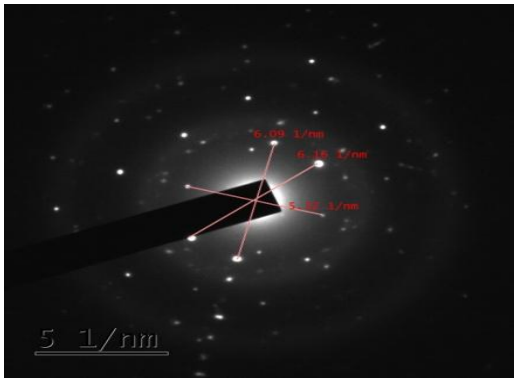
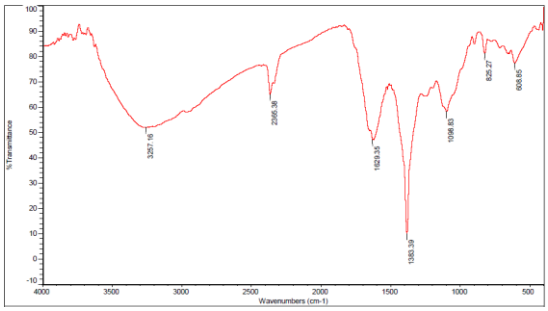
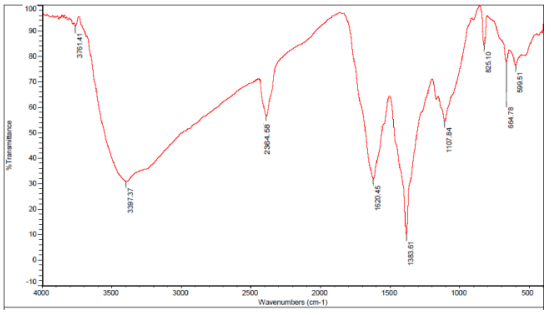


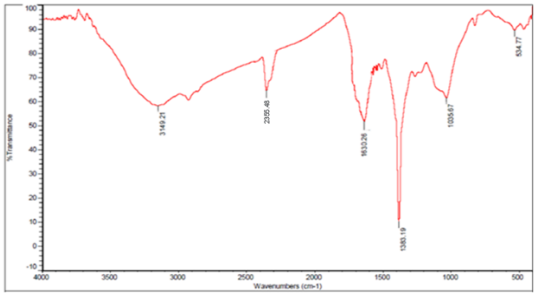
Figure 7



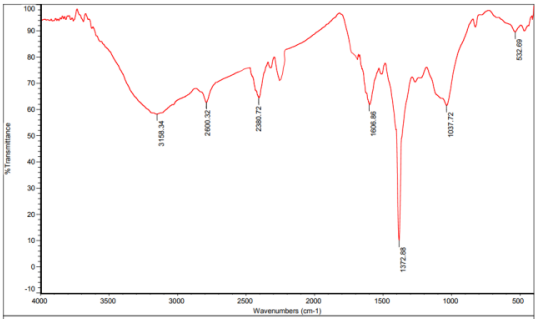
(CCAg)



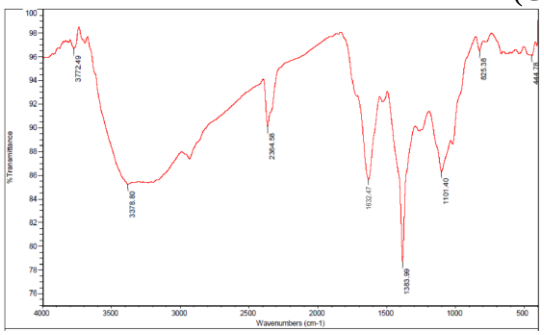
(APAg)



(MMAg)

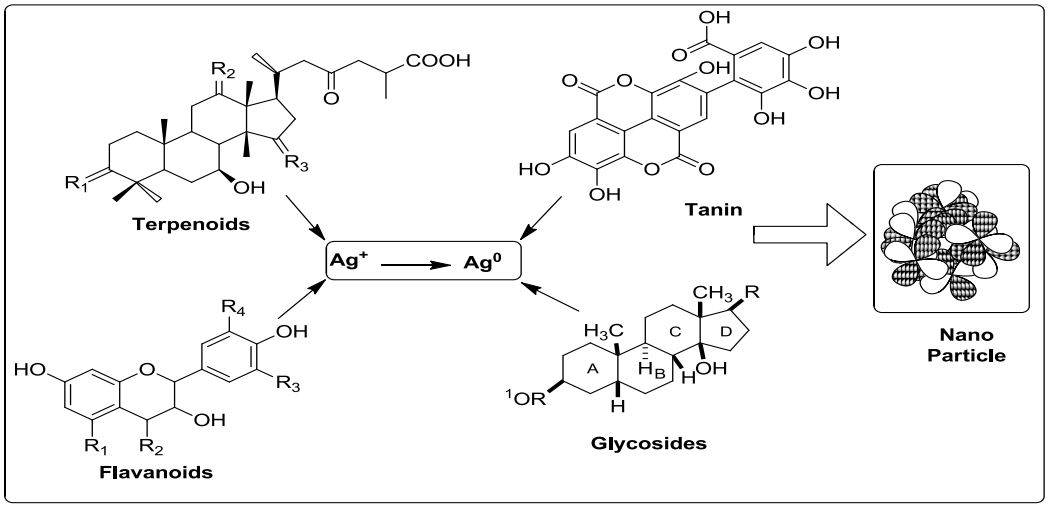


(CFAg)



(CCAg)

Figure 8



Scheme 1

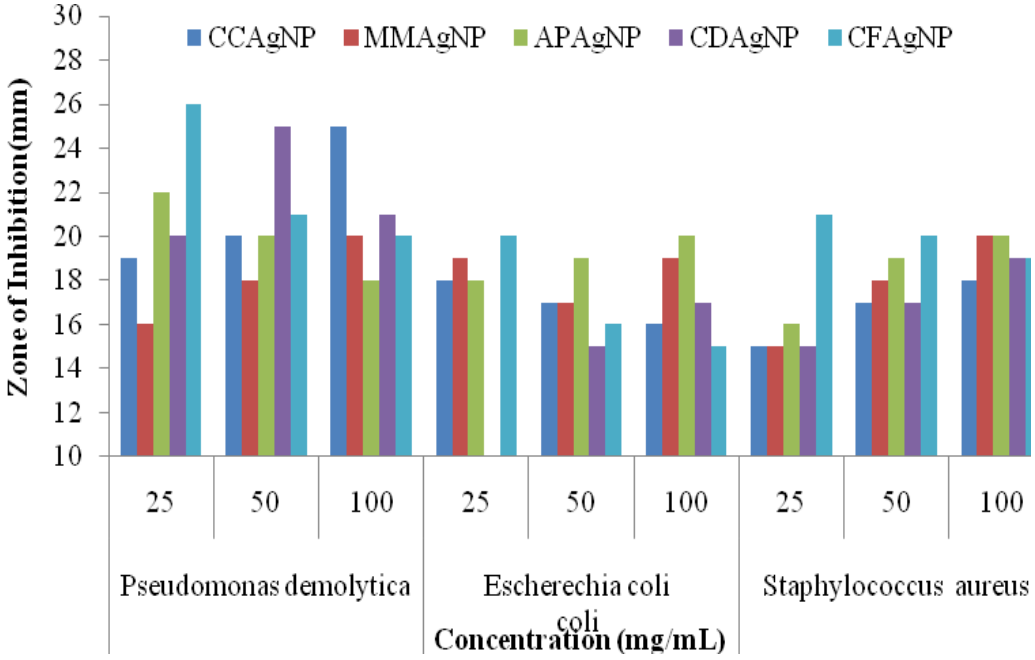


Figure 9

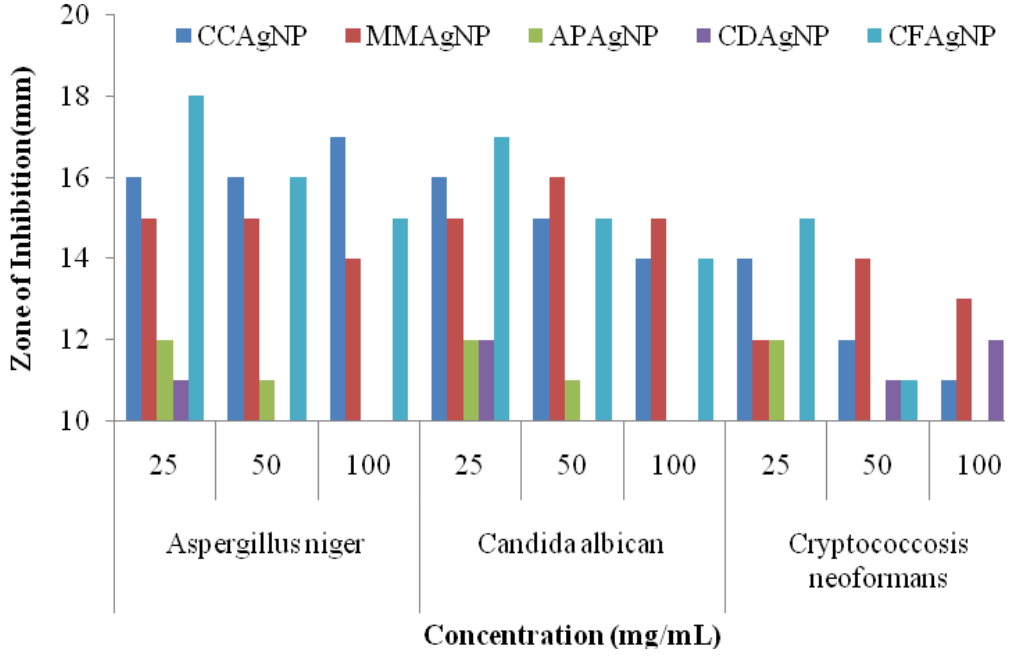
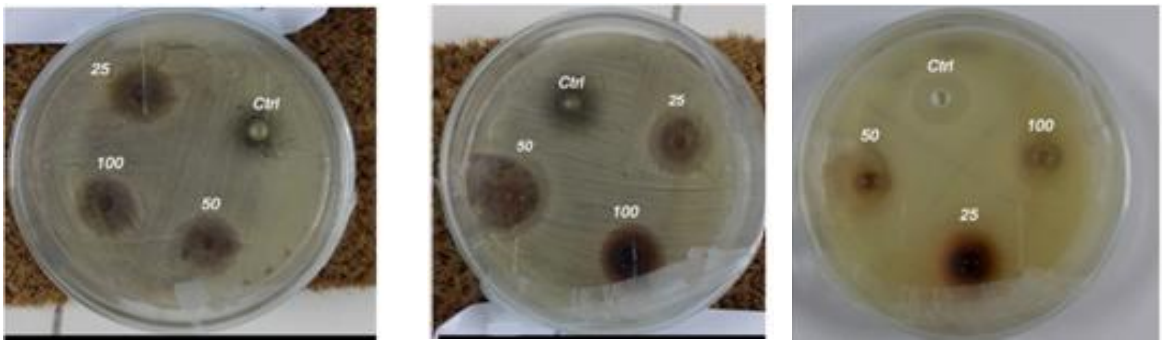
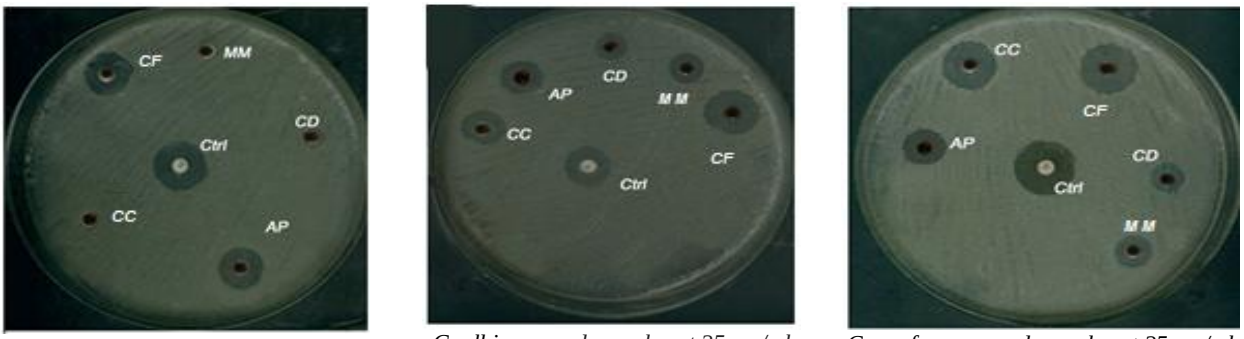


Figure 10



(a) (b) (c)

Figure 11



A. niger and samples at 25 mg/ml *C. albicana and samples at 25 mg/ml* *C. neoformans and samples at 25 mg/ml*

Figure 12

Table 2

	Zone of Inhibition (mm) against pathogenic Bacteria								
Samples	***Pseudomonas demolytica***			***Escherechia coli***			***Staphylococcus aureus***		
	25 mg/ml	**50 mg/ml**	**100 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**
CCAgNP	19	20	25	18	17	16	15	17	18
MMAgNP	16	18	20	19	17	19	15	18	20
APAgNP	22	20	18	18	19	20	16	19	20
CDAgNP	20	25	21	10	15	17	15	17	19
CFAgNP	26	21	20	20	16	15	21	20	19

Table 3.

Samples	Zone of Inhibition (mm) against pathogenic Fungi								
	Aspergillus niger			***Candida albicana***			***Cryptococcosis neoformans***		
	25 mg/ml	**50 mg/ml**	**100 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**	**25 mg/ml**	**50 mg/ml**	**100 mg/ml**
CCAgNP	16	16	17	16	15	14	14	12	11
MMAgNP	15	15	14	15	16	15	12	14	13
APAgNP	12	11	10	12	11	10	12	10	07
CDAgNP	11	10	09	12	10	05	10	11	12
CFAgNP	18	16	15	17	15	14	15	11	10

Assessment of antimicrobial activity of bio synthesized AgNPs

The antimicrobial activities of colloidal AgNPs are prejudiced by the dimensions of the particles. The smaller particles lead to the greater antimicrobial effects [46]. The diameter of inhibition zones (mm) around each well with silver nanoparticles solution is represented in Figure 9, 10 and Table 2, 3. In the present study, the natural bio extracts against microbes such as *Pseudomonas demolytica, Escherechia coli* and *Staphylococcus aureus, Aspergillus niger*, *Candida albicans* and *Cryptococcus neoformans* were evaluated.

The total activities of bio extracts against bacterial and fungal indicated that CFAgNPs was found to be most active at lower concentration (25mg/ml) against *P. demolytica* (26 mm) and S. aureus (21mm) and *Escherechia coli* (20 mm) respectively as it has been synthesized to 2nm in size. The maximum inhibition zone by Gentamycin as positive control on *Pseudomonas demolytica* (28mm), *Escherechia coli* (24mm), *Staphylococcus aureus* (23mm). Silver nitrate solution was used as negative control. The maximum anti-fungal activity was exposed by CFAgNPs against *A. niger* (18mm), *C. albicana* (17mm) and *C. neoformans* (15mm) at lower concentration respectively. The other extracts of different families have demonstrated activity against several test microorganisms. Fluconazole was used as Positive control. Three replicates were maintained and the mean diameter value was expressed in millimeters. The antimicrobial activity of aqueous extract of CFAgNPs may be due to the existence of poly phenolic group which may destroy the lipid membrane of microbial cell. Table 2 & 3 shows the effect of all AgNPs synthesized against microbial strains.

Literature reports on phytochemical, pharmacological and biological investigations of *Cocusnucifera* fibres /dust extracts and *Abutilon palmeri* are scarce.

Conclusion

A facile one-pot synthesis of silver nanoparticles using various bio extracts at 70ºC temperature is reported in this study. It proves 100% green synthesis, cost-effective, efficient and eco-friendly competent inters of reaction time and stability of the synthesized nanoparticles. UV–Vis spectrophotometer, XRD, Particle size analyser, Fluor spectroscopy, SEM and TEM techniques have confirmed the reduction of silver nitrate to silver nanoparticles. The zones of inhibition that were created in the antimicrobial screening test showed, that the synthesized Ag NPs has the efficient antimicrobial activity against a choice of microbials. Thus among biosynthesized AgNPs of all extracts, CFAgNPs is expected to have prospective applications in biomedical uses such as antimicrobial treatment and will play a vital role in near future.

C. nucifera fibres/dust aqueous extracts was selected for further work to isolate, identify the bioactive and characterize the anticancer compounds in *invitro* and *invivo* studies. A parallel study will be undertaken to compare the antibacterial activity of *Cocusnucifera fibres/ dust* aqueous extracts with different nano metal oxides.

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