American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

Low Cost and Ecofriendly Phytosynthesis of Silver Nanoparticles Using *Cassia roxburghii* Stem Extract and its Antimicrobial and Antioxidant Efficacy

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Date of Receipt- 09/08/2014
Date of Revision- 22/08/2014
Date of Acceptance- 27/08/2014

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ABSTRACT

A new and novel approach for the green synthesis of silver nanoparticles is the need of the hour. Use of plant parts for the synthesis of silver nanoparticles is ecofriendly, economic and cost effective. In the present paper, silver nanoparticles were synthesized using aqueous stem extract of *Cassia roxburghii* DC as a reducing agent. The biosynthesized AgNPs were characterized by various spectral analysis like UV–Vis, FTIR, XRD, TEM and Zeta potential measurement. UV–vis spectra showed maxima absorption peak at 432 nm. XRD and TEM analysis revealed AgNPs to be face-centered, cubic structures spherical in shape with an average particle size of 32-35 nm.

The synergistic antibacterial activity was evaluated against two Gram positive, two Gram negative bacteria and four fungi with fifteen commercial antibiotics alone and antibiotics plus synthesized AgNPs. Antioxidant activity of AgNPs was evaluated by ABTS and FRAP assay. The AgNPs showed synergistic antibacterial activity even better than some antibiotics and also good antioxidant activity. The results suggest that *Cassia roxburghii* stem could be exploited for the fabrication of AgNPs with potential therapeutic application in nanomedicine especially against multi drug resistant microorganisms which are cost effective and ecofriendly and simple. They can be definitely used in cosmetics, medical and pharmaceutical applications

Keywords: *Cassia roxburghii*, Green synthesis, Silver nanoparticles, Spectral analysis, Antimicrobial.

INTRODUCTION

There are no qualms that antimicrobial agents have safeguarded the humanity from torment of infectious diseases. Mankind has faced the dangerous attacks of microorganisms and tried to endure it with the use of antibiotics or antimicrobial agents. However, multidrug-resistant bacteria are becoming more common and they are frequently resistant to almost all the current antibiotics. Many biochemical and physiological mechanisms may be responsible for resistance[1].Drug-resistant bacterial infections because considerable patient mortality and morbidity and rising antibiotic resistance is seriously threatening the vast medical advancements made possible by antibiotics over the past years[2].The need of the hour suggests that new approaches are required to combat emerging infections and the global spread of drug-resistant bacterial pathogens[3,4].Free radicals are extremely reactive species that cause oxidative damage to various biomolecules like lipids, proteins, DNA in human body and are responsible for a number of pathologies like cardiovascular diseases, diabetes, cancer, Parkinson's disease, Alzheimer's disease, acquired immunodeficiency syndrome, Huntington's disease and many other chronic diseases[5,6]. Antioxidants are compounds that react with free radicals and prevent undesirable oxidation processes. Unlike synthetic drugs, antimicrobials and antioxidants of natural origin from plants are not associated with any side effects and that is why they are becoming more and more popular. The plant extracts provide ample opportunities for new drug discoveries because of the enormous diversity present in them[7]. All plants have same or different secondary metabolites which are responsible for its biological activity[8,9].

The *Cassia* genus belonging to the family Fabaceae represents one of the large and most diverse group of flowering plants including herbs and trees. They are widely distributed in most tropical and subtropical countries. Cassia species have biological and pharmacological activities and have many medicinal uses in traditional system of medicine. They are reported for antimicrobial and antioxidant activity[10], nephroprotective activity[11], antidiabetic activity[12], hepatoprotective activity[13], immunomodulatory activity[14] etc. *Cassia roxburghii* is one of the medicinal plants used in ethnomedicine for the treatment of various liver ailments[15].The therapeutic applicability of silver and medicinal plants in treating bacterial infections is well known[16, 17]. Silver nanoparticles have diverse application like molecular diagnostics, catalysis, drug delivery [18,19] including antimicrobial and antioxidantproperties[20,21] .

Silver nanoparticles (NPs) can be synthesized by various chemical and biological methods. Biological method include use of enzyme, microorganism or plant extracts but the later one is preferred because plant extracts act as reducing and stabilizing agent and they also influence the shape and size of the synthesized NPs[22].This is because different extracts contain different concentrations and combinations of phytoconstituents[23,17]; different parts of the same plant (stem, flower, leaf) may influence differently the shape and size of NPs synthesized. Silver NPs can be prepared from any part of the plant like plant extract *Cleistanthuscollinus*[24], fruit *Piper longum*[25], rind *Bruceajavanica*[26], leaf *Tribulu-sterrestris*[27], stem *Shoreatumbuggaia*[28], etc.

Amongst different parts of the plant used for the synthesis of NPs leaf is used very frequently while stem and flowers are less attempted. Hence in the present work, stem of *C. roxburghii* is used for the synthesis of silver NPs. The

characterization was done by various spectral analyses like UV-Vis, FTIR, XRD, TEM and Zeta potential measurement. The synthesized silver NPs were evaluated for their synergistic antimicrobial activity with fifteen commercial antibiotics against four pathogenic bacteria and four fungi which included one clinical isolate. The antioxidant capacities of the synthesized silver NPs were also checked by using ABTS and FRAP antioxidant assays.

MATERIALS AND METHODS

Chemicals

Fresh young stem of *Cassia roxburghii* DC was collected from Saurashtra University campus, Rajkot Gujarat, India. All the chemicals were obtained from Hi Media Laboratories and Sisco research Laboratories Pvt. Limited, Mumbai, India. Ultra purified water was used for experiment.

Preparation of the extract

Fresh stem was thoroughly washed with tap water, followed by double distilled water and cut into small pieces. 5 g of cut stem pieces were boiled for 5 min in 100 ml ultra-pure water and filtered through Whatmann No. 1 filter paper. The filtered *C. roxburghii* stem extract was used for the synthesis of silver NPs.

Synthesis of silver NPs

Aqueous solution (1mM) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver NPs. 6ml of extract was added to 40 ml of 1 mM $AgNO_3$ solution for the reduction of Ag^+ ions. The synthesis of silver NPs was carried out at room temperature (25°C + 2°C) for 24 h in dark.

Effect of boiling time

In order to standardized the effect of boiling time for the preparation of aqueous extract of *C. roxburghii*, 5g of cut stem pieces were taken in 100 ml ultra-pure water and boiled it for 5 min, 10 min and 15 min. and then filtered through Whatmann No. 1 filter paper. The filtered *C. roxburghii* stem extract was used for the synthesis of silver NPs.

Effect of extract amount

In order to standardized the effect of extract amount to be added to 1 mM $AgNO_3$ solution, different amount of extract (1.5 ml, 3.0 ml, 4.5 ml and 6.0 ml) was added to $AgNO_3$ solution. The formation of AgNPs was monitored as a function of time of reaction on a spectrophotometer by taking O.D. at 440 nm at an interval of 15 min.

Characterization of the synthesized silver NPs

Synthesis of silver NPs solution with stem extract may be easily observed by ultraviolet – visible (UV-Vis) spectroscopy. The reduction of the Ag^+ ions in solution was monitored by periodic sampling of aqueous component and measuring the UV-Vis spectra of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on a spectrophotometer (Shimadzu UV-1601) in 400-700 nm range operated at a resolution of 10 nm.

FTIR analysis of silver NPs

Possible functional groups involved in the synthesis and stabilization of silver NPs was studied by FTIR spectroscopy. The FTIR was recorded in the range of 400-4000 cm^{-1} NicoletIS10 (Thermo Scientific, USA) The various modes of vibrations were identified and assigned to determine the different functional groups present in the *Cassia* stem extract.

Zeta Potential Measurement

Zeta potential is an essential parameter for the characterization of stability in aqueous nano suspensions. The zeta

potential measurement was performed using a Microtra (Zetatra Instruments).

XRD measurement

The silver NPs solution thus obtained was purified by repeated centrifugation at 10000 rpm for 10 min followed by redispersion of the pellet of silver NPs into Acetone. After air drying of the purified silver particles, the structure and composition were analyzed by XRD. The dried mixture of silver NPs was collected for the determination of the formation of Ag NPs by an X'Pert Pro x-ray diffractometer (PAN analytical BV) operated at a voltage of 40 kV and a current of 30mA with Cu Kα radiation in θ- 2 θ configurations. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula. $D= 0.94 \lambda / \beta \cos \theta$ where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle.

TEM analysis

TEM analysis was done to visualize the shape as well as measure the diameter of the biologically synthesized silver NPs. The sample was dispersed in double distilled water. A drop ofthin dispersion was placed on a "staining mat". Carbon coated copper grid was inserted into the drop with the coated side upwards. After about ten minutes, the grid was removed and air dried. Then screened in JEOL JEM 2100 Transmission Electron Microscope.

Antimicrobial activity

The antimicrobial activity of AgNPs with 15 commercial antibiotics and antibiotics alone was determined against 2 Gram positive bacteria (*Staphylococcus aureus* ATCC NO 29737and*Bacillus cereus* ATCC NO 11778) and 2 Gram negative bacteria (*Escherichia coli* NCIM NO 2931and *Pseudomonas aeruginosa* ATCC NO.27853) and 4 fungal (*Candidaalbicans* NCIM NO 3102, *Candidaglabrata* NCIM NO 3448, *Cryptococcaeneoformans* NCIM NO 3542 and No.44, a clinical isolate candida, obtained from Spandan diagnostic center, Rajkot, Gujarat, India) strains, by using agar disc diffusion method[29]. All the microorganisms were obtained from NCL, Pune, India.

Determination of antioxidant activities

ABTS assay

The ABTS cation radical scavenging activity of silver NPs was measured by the method as described by[30]. ABTS radical cations are produced by reacting ABTS (7 mM) and potassium persulfate (2.45 mM) and incubating the mixture at room temperature in dark for 16 h. The ABTS working solution obtained was furthure diluted with methanol to give an absorbance of 0.85±0.20 at 734 nm. 1.0 ml of different concentration (1to 1000 µg ml^{-1}) of silver NPs and fractions diluted by methanol was added 3.0 ml of ABTS working solution. The reaction mixture was incubated at room temperature for 5 min, and then the absorbance was measured at 734 nm using a UV-Vis (Systronics Spectrophotometer) against a blank sample. Ascorbic acid (1 to 10 µg ml^{-1}) was used as a positive control. Percentage of inhibition was calculated using the formula (C-T/C *100).

FRAP assay

The reducing ability of AgNPs was determined by FRAP assay[31]. FRAP assay is based on the ability of antioxidants to reduce Fe^{+3} to Fe^{+2} in the presence of TPTZ, forming an intense blue Fe^{+2} – TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). 0.1 ml extract is added to 3.0 ml FRAP reagent [10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10 mM TPTZ (2, 4, 6- tripyridyl-s-

triazine) in 40 mM HCL and 1 part 20 mM $Fecl_3$] and the reaction mixture is incubated at 37 °C for 10 min and then the absorbance was measured at 593 nm. $FeSO_4$ (100 to 1000 µM ml^{-1}) was used as positive control. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed at M $FeSO_4$ equivalents per gram of extracted compound.

RESULTS

Standardization

Effect of boiling time

In order to evaluate the effect of boiling time for the *C. roxburghii* stem extract preparation, the stem was boiled for 5, 10 and 15 min. The UV-Vis spectrum of silver NPs was recorded at different time interval of reaction medium. There was slight difference in the formation of AgNPs by 5, 10 or 15 min boiling time but best was 5 min boiling time (Fig. 1B). Hence 5 min boiling time was fixed for the preparation of aqueous extract of stem of *C. roxburghii*.

Effect of extract amount

There was a clear increase in AgNPs formation with increase in amount of extract added (Fig. 1C). Addition of 6.0 ml of extract showed substantial increase in the formation of AgNPs, so it was taken for the synthesis of NPs.

Characterization

As soon as stem extract (a very light brownish solution) was added to colour less silver nitrate solution, the light brownish solution turned to brown colour and the intensity of the colour increased with time and finally turned to dark brown (Fig. 2A).

UV-visspectral analysis

The UV-visible spectra of silver NPs was recorded at different time interval of the reaction medium (0, 30, 40, 60, 120 min) using *C. roxburghii* stem extract with 1 mM $AgNO_3$ (Fig. 2B). Maximum absorption peak was at 432 nm (Fig. 2B).The peak intensity increased with time indicating increase in the concentration of synthesized NPs.

FTIR spectral analysis

FTIR analysis was done to identify the possible bio-reducing biomolecules in the stem extract. The spectra of AgNPs revealed strong bands at 3527.92, 2339.73, 1264.38, 966.37, 830.38, 754.19 and 667.39 respectively (Fig. 3A). The intense bands at 3527.92 cm−1 are characteristic group of primary O-H stretching of alcohols, phenols. The peak at 1264.38 cm−1 corresponds to C-N, C-O stretch of aromatic amines. The peak of 966.37 cm−1 corresponds to C-H wag (-CH_2X) alkylhalides compound. The peaks of 830.38, 754.19, 667.39 are assigned to the stretching of C-Cl, C -Br respectively of alkyl halides group.

Zeta potential

Zeta potential is an essential parameter for the characterization of stability in aqueous nano suspensions. The zeta potential of *C. roxburghii* stem AgNPs was -5.98 mv (Fig. 3B) suggesting that the surface of the NPs was negatively charged that dispersed in the medium.

X-ray diffraction (XRD)

XRD analysis showed four distinct diffraction peaks at 2 theta values of 17.029, 10.613, 27.23 and 12.81 indexed to the (1 1 1), (2 0 0), (2 2 0) and (311) crystalline planes of the fcc structure of metallic silver (lattice Constant a = 4.086 A◦, was matched well with Joint Committee on Powder Diffraction Standards (JCPDS) values) (Fig.4).

TEM analysis

The optical signature of AgNPs was elucidated in terms of the distribution of sizes and shapes observed by transmission electron microscopy (TEM) images. A drop of silver NP solution was placed on to a carbon coated Cu grid and the sample was allowed to dry. The TEM images were recorded at different magnification to find the individual particles. TEM of AgNPs synthesized using *C. roxburghii* stem clearly showed that the silver NPs were spherical in shape. The average size of the AgNPs was in the range of 32-35 nm (Fig. 5A, B, C). The size and selected area electron diffraction (SAED) pattern of the AgNPs synthesized using *C. roxburghii* stem extract was recorded confirming the crystalline nature of AgNPs (Fig. 5D).The inset in each pattern showed the respective selected region. The presence of bright circular rings in the SAED patterns confirmed the crystalline nature of the silver NPs. The spots corresponding to various orientations appearing inside the concentric rings also showed that the obtained silver NPs had a good crystallinity. Energy-dispersive (EDX) spectroscopy analysis was performed to see the presence of elemental silver (Fig. 5E). The strong signal in the silver region confirmed the formation of AgNPs. The peaks for copper and carbon were also found but these were originated from the carbon-coated copper grid used for TEM sample preparation and EDX analysis (Fig. 5E).

Antimicrobial activity

In the present work, 15 commercial antibiotics were tested alone and with AGNPs against 2 Gram positive, 2 Gram negative and 4 fungi which included 1 clinical isolate. The diameter of inhibition zone and increase in fold area for the entire test organisms was measured. The antibacterial activity of AgNPs with antibiotics was better than antibiotics alone against almost all the tested bacterial strains (Tables 2).Out of 11 antibiotics tested AgNPs showed more activity than 8-9 antibiotics against both Gram negative bacteria *E. coli* and *P. aeruginosa*; maximum increase was against *E. coli* (1.25).It was observed that CFP^{30} and TE^{30}had highest increase in the fold area against *E. coli* and *P. aeruginosa* respectively (Table 2a). *B. cereus* was more susceptible than *S. aureus* among the two Gram positive bacteria; maximum increase was that of CC^{10} followed by CEP^{30} against *B.cereus*. Antifungal activity was moderate though highest increase in fold area was 1.08 by FLC^{10} against *C. neoformans* followed by KT^{30} against *C. albicans*. Clinical isolate 44 showed poor antifungal activity (Table 2b).

Antioxidant activity

ABTS scavenging activity showed increase in free radical scavenging activity which increased with the increase in the concentration of the NPs (Fig. 6). The IC_{50} values of *cassia* stem AgNPs was 90 µg/ml. FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). Ferric reducing antioxidant power (FRAP) of AgNPs was 2.20 (Mg^{-1}).

DISCUSSION

Synthesizing silver NPs by the use of plant extracts is ecofriendly, economic and non-hazaradous and this is green approach. In the present study, an attempt was made to synthesize silver NPs from C. *roxburghii* stem extract. For synthesis of silver NPs optimization or standardization of boiling time for aqueous extract preparation of stem of *C. roxburghii* and amount of extract to be added to silver nitrate solution was an important step for the biosynthesis of AgNPs. Zayed *et al* [32] also reported that amount of

plant extract played a critical role in AgNPs formation.

The first indication of silver NPs formation from plant extract is visual i.e. the appearance of a yellow is brown color due to the excitation of surface plasmon vibrations[33]. The formation of silver NPs varies from plant to plant as evidenced from the colour change of the aqueous solution reported by Moteriya *et al* [34]. It takes from few minutes to hours as reported by others Lukman *et al*. 2011, Shameli *et al* [35] and Chanda[36.] In the present work, the colour change from slight brownish colourto brown was within 15 min indicating the formation of NPs; the intensity steadily increased up to 2 h and it turned dark brown within 24 h. Similar results were reported by Christensen *et al* [37].

UV–Vis spectroscopy is a valuable tool to observe the formation of NPs in aqueous solution. UV-spectra revealed maximum absorption peak at 432. The peak intensity increased with time indicating increase in the concentration of synthesized NPs. Philip[38]reported similar results for AgNPs synthesized from *Mangifereindica* leaf extract. Mallikarjuna *et al*.[39] and Sathish kumar *et al*. [40] reported maximum absorption peak of sliver NPs at 436 and 435 from *Ocimum sanctum* and *Cinnamon zeylanicum* extracts respectively.

In order to identify the possible biomolecules present in *C. roxburghii* stem which are responsible for the reduction of silver and its stabilization, FTIR measurement were carried out.

There were many functional groups present and these functional groups represent phytoconstituents like flavonoids, triterpenes and alkaloids of the stem which probably had an effective role in the green synthesis of silver NPs and also may be responsible for capping and stabilization of the synthesized NPs (Table 1). The flavonoids present in the stem extract are powerful reducing agents which may be suggestive for the formation of AgNPs by reduction of silver nitrate. The carboxylate group presentin proteins can acts as surfactant to attach on the surface of NPs and results in AgNPs stabilization. Thus *C. roxburghii* stem extract may play a dual role as stabilizing and reducing agents of Ag NPs. Similar results were reported in *T. purpurea* leaf [41].But, the exact mechanism is unclear and needs further investigation.

The zeta potential of C. *roxburghii* stem AGNPs was -5.98 mv. The negative charge on the surface of the synthesized AgNPs can cause strong repulsive force among particles which may prevent from aggregation. Hence, it can be concluded that the synthesized NPs are fairly stable. The phytoconstituents present in the stem extract may be responsible for stabilizing the synthesized NPs.

The sharp peak in XRD analysis clearly indicated the crystalline nature of the synthesized AgNPs. TEM analysis revealed that *C. roxburghii* stem AgNPs were spherical in shape. The average size of the AgNPs was in the range of 32-35 nm. The spherical shape of NPs was reported in *Piperpedicellatum* extract[42] and *Hibiscus cannabinus* extract[43]. The shape and size of NPs formed varies from plant to plant and part used and also the phytoconstituents present in them at the time of synthesis. Similar results were reported by Gengan *et al*[44] Kumar *et al* [45].The sharp signal peak of silver strongly indicated the reduction of sliver ion by *C. roxburghii* stem in to elemental sliver. TEM images showed that the surfaces of the AgNPs were surrounded by a black thin layer of some material which might be due to the capping organic constituents of stem extract. Khan *et al*. [46] and Satish kumar *et al*.[47] also reported that synthesized AgNPs were surrounded by a capping material present in the plant extract.

In the present study, the antimicrobial activity of synthesized AgNPs and 15 commercial antibiotics was tested against bacteria and fungi individually and in

combination i.e. Antibiotics alone and AgNPs plus antibiotics. The diameter of zone of inhibition and increase in fold area for all the bacterial and fungal strains was measured. Increase in fold area can give an idea about the synergistic activity of the compounds tested against the bacterial or fungal strains. Increase in fold area was more against Gram negative bacteria than Gram positive bacteria. Similar results were reported by Antony *et al*[48]. Thakur *et al*.[49] reported antibacterial activity against *P. aeruginosa* by *Acacia Arabica* gum AgNPs. Niraimathi*et al*.[50]reported antibacterial activity of AgNPs against *S. aureus* and *E. coli*. Antifungal activity was moderate though highest increase in fold area was 1.08 by FLC^{10} against *C. neoformans* followed by KT^{30} against *C. albicans*. Antifungal activity of AgNPs with commercial antibiotics is reported by Lee *et al*.[51] and Kotakadi *et al*.[52]. We are perhaps for the first time reporting synergistic effect of 15 antibiotics with AgNPs against pathogenic microorganisms and it is a new finding.

Antioxidant property was evaluated using ABTS radical cation scavenging assay and FRAP assay. $ABTS^{\bullet+}$ is a blue chromophore generated from the oxidation of ABTS by potassium persulfate, in the presence of the plant extract, preformed cation radical gets reduced and employs a specific absorbance at 734 nm, a wavelength remote from the visible region, and it requires a short reaction time.Ferric reducing antioxidant power (FRAP) of AgNPs was 2.20 ($M\ g^{-1}$). Abdel-Aziz *et al*.[53] also reported antibacterial and antioxidant activity of silver NPs synthesized from *Chenopodiummurale* leaf extract.

In the present work, silver NPs were successfully synthesized using *Cassia rohburghii* stem extract. The method is simple, ecofriendly and efficient. The average size of the NPs was in the range of 32-35 nm and they were spherical in shape. The synthesized AgNPs showed very good synergistic antibacterial activity i.e. antibiotics plus AgNPs showed more inhibitory activity than antibiotics alone and also good antioxidant activity. Thus, these ecofriendly silver NPs can be used as an excellent antimicrobial agent against multi drug resistant pathogenic microorganisms and also can be a good antioxidant agent to tackle oxidative stress related disorders. However, more work especially *in vivo* studies are required and studies in this direction are in progress.

ACKNOWLEDGEMENTS

The authors thanks Prof. S.P. Singh, Head, Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India for providing excellent research facilities. We acknowledge the support extended by Prof. Shipra Baluja, Department of Chemistry and Prof. D. G. Kuberkar, Department of Physics, Saurashtra University for FTIR and XRD analysis of the samples.

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Table 1. Phytochemicals test of *C. roxburghii* stem powder

Test	Result
Flavanoids	+++
Tannins	-
Phlobatanins	-
Triterpenes	+++
Steroids	-
Saponins	-
Cardiac glycoside	-
Meyer's	++
Dragondroff	+++
Wagners	++
Legal's	-

Table 2a. Synergistic activity of AgNPs of *C. roxburghii* stem with different standard antibiotics against Gram negative and Gram positive bacteria

***Escherichia coli* (NCIM NO 2931)**				***Pseudomonas aeruginosa* (ATCC NO 27853)**			***Bacillus cereus* (ATCC NO 11778)**			***Staphylococcus aureus* (ATCC NO 29737)**		
Anti biotic	Anti biotic (A)	Anti biotic + AgNPs(B)	Increase In fold area	Anti biotic (A)	Anti biotic + AgNPs(B)	Increase In Fold area	Anti biotic (A)	Anti biotic + AgNPs (B)	Increase In fold area	Anti biotic (A)	Anti biotic + AgNPs (B)	Increase In fold area
AMP	9	10	0.23	23	26.5	0.32	-	-	-	33	35	0.12
PB^{100}	9	10	0.23	13.5	16.5	0.49	11	12.2	0.23	9	10.5	0.36
Gen^{10}	13.5	17	0.58	22	25	0.29	19.5	22.2	0.29	16	17.5	0.19
C^{30}	25.5	26.5	0.07	14	16	0.30	33	33	0	24	27	0.26
P^{10}	-	-	-	14	16	0.30	-	-	-	33	33	0
Ak^{10}	17.5	21	0.44	24.5	29	0.40	22	22	0	17	18.5	0.18
TE^{30}	22	26	0.28	26.5	35	0.74	27.5	30	0.19	28	31	0.22
CEP^{30}	10	10	0	11	11	0	13	18	0.91	37	37	0
AMC^{10}	9	9	0	20.5	25.5	0.54	-	9		31.5	35	0.23
CFP^{30}	9	13.5	1.25	34	37.5	0.21	13.5	16	0.40	24	25.5	0.12
CC^{10}	12	16	0.78	-	-	-	14	19.5	0.94	11.5	13.5	0.37

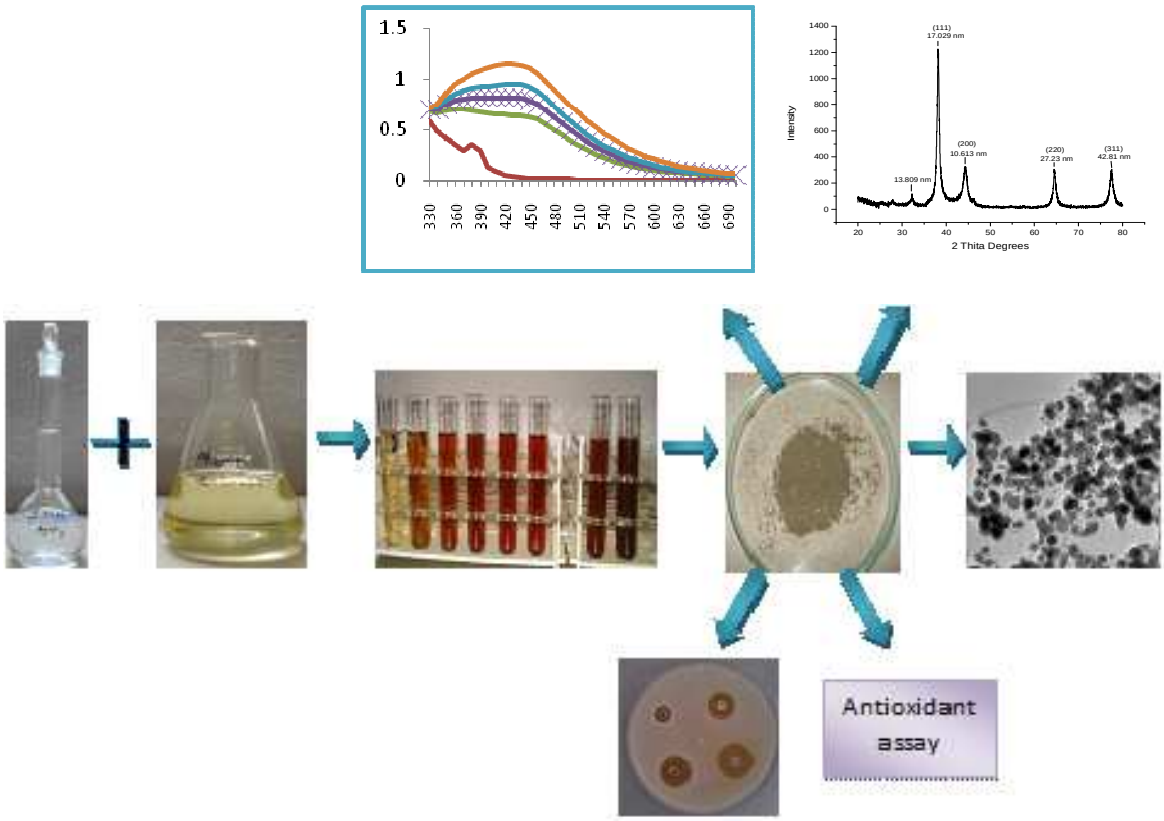
AMP – Ampicillin, PB^{100}- Polymyxin, Gen^{10} – Gentamicin, C^{30} – Chloramphenicol, P^{10} – Penicilli-G, AK^{10} – Amikacin, TE^{30} – Tetracycline, CEP^{30} – Cephalothin, AMC^{10} – Amoxyclav, CFP^{30} – Cefpirome, CC^{10} – Clotrimazole

Mean surface area of the inhibition zone was calculated for each from the mean diameter. Increase in fold area was calculated as $(B^2 – A^2)/A^2$, where A and B are the inhibition zones for Ag-NPs and antibiotics + Ag-NPs, respectively.

Table 2b. Synergistic activity of AgNPs of *C.* stem with different standard antibiotics against fungi

***Candida glabrata* (NCIM NO 3448)**				**44**			***Candida albicans* (NCIM NO 3102)**			***Cryptococcae neoformans* (NCIM NO 3542)**		
Anti fungal	Anti fungal (A)	Anti fungal + AgNPs (B)	Increase in fold area	Anti fungal (A)	Anti fungal + AgNPs(B)	Increase in fold area	Anti fungal (A)	Anti fungal + AgNPs(B)	Increase in fold area	Anti fungal (A)	Anti fungal + AgNPs(B)	Increase in fold area
NS^{100}	29	31	0.14	21	21.5	0.04	17	19	0.24	23	23	0
KT^{30}	24.5	30	0.49	-	-	-	15	19.5	0.69	15.5	19.5	0.58
FLC^{10}	21.5	23	0.14	-	-	-	20.5	23.5	0.31	13.5	19.5	1.08
AP^{100}	15	15	0	12	12.5	0.08	10.5	11	0.09	12	12	0

NS^{100} – Nystatin, KT^{30}- Ketoconazole, , FLC^{10} – Fluconazole, AP^{10} – Ampotericin ; Mean surface area of the inhibition zone was calculated for each from the mean diameter; Increase in fold area was calculated as $(B^2 – A^2)/A^2$, where A and B are the inhibition zones for Ag-NPs and antibiotics + Ag-NPs, respectively.



Green synthesis of silver nanoparticles using *C. roxburghii* stem extract and $AgNO_3$. Characterization by spectral analysis like UV-Vis, TEM, FTIR and XRD. Evaluation of antimicrobial and antioxidant potential

Figure 1(A). *Cassia roxburghii* DC Plant

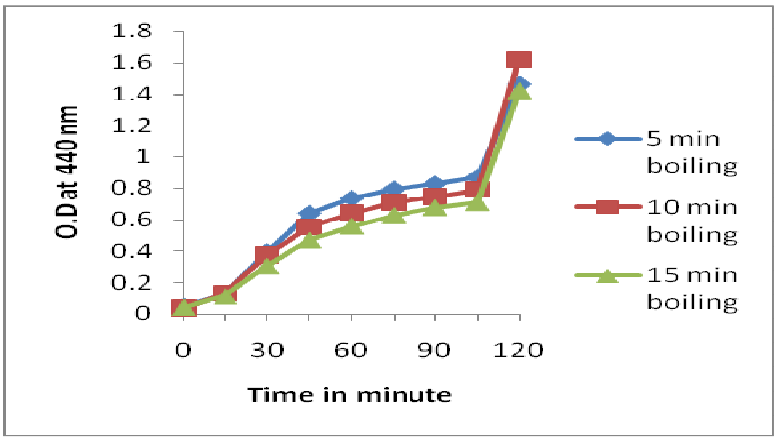


Figure 1(B). Effect of boiling time

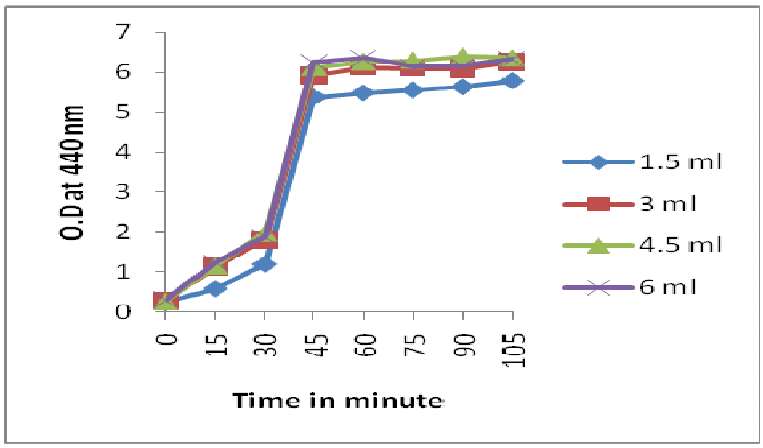


Figure 1(C). Effect of extract amount

Figure 2(A). Color change in the reaction mixture within 2 h

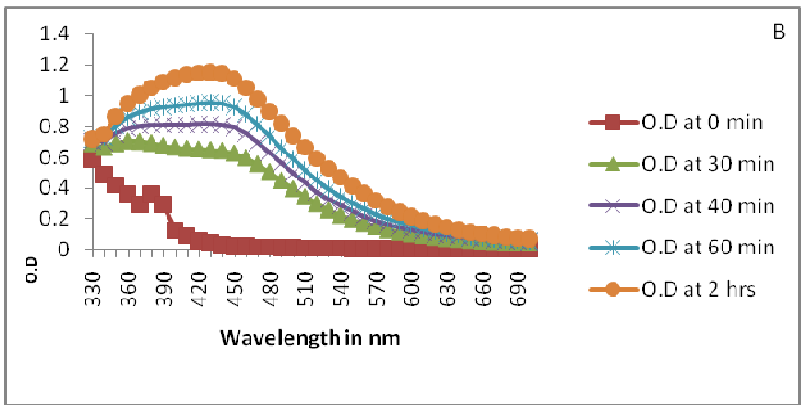


Figure 2(B). UV-visible spectrum of biosynthesized CR-AgNPs at different time interval, showed peak at 432 nm

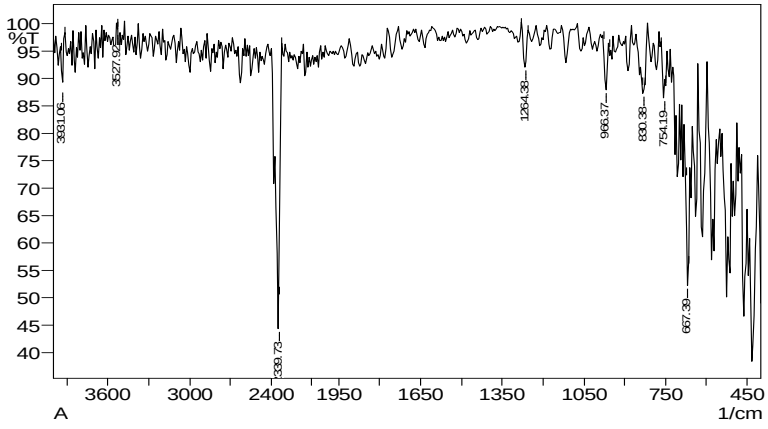


Figure 3(A). FTIR spectrum of biosynthesized CR AgNPs

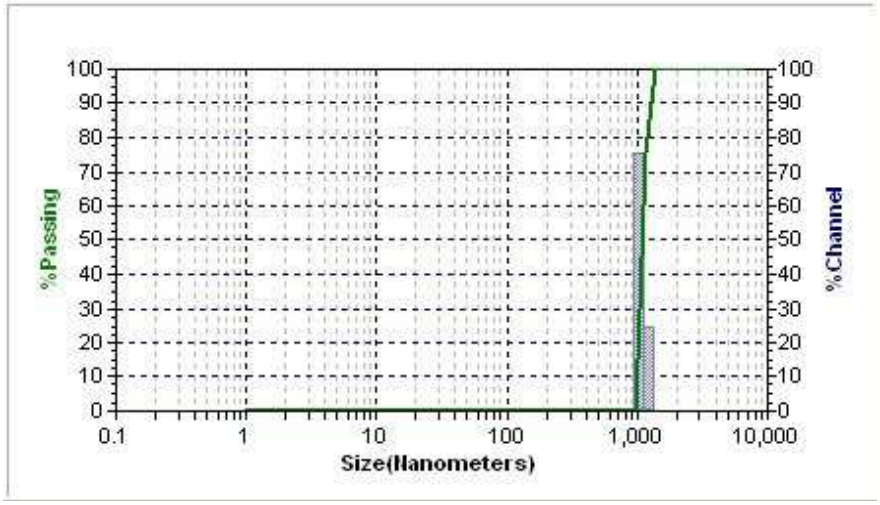


Figure 3(B). Zeta potential of biosynthesized CR AgNPs

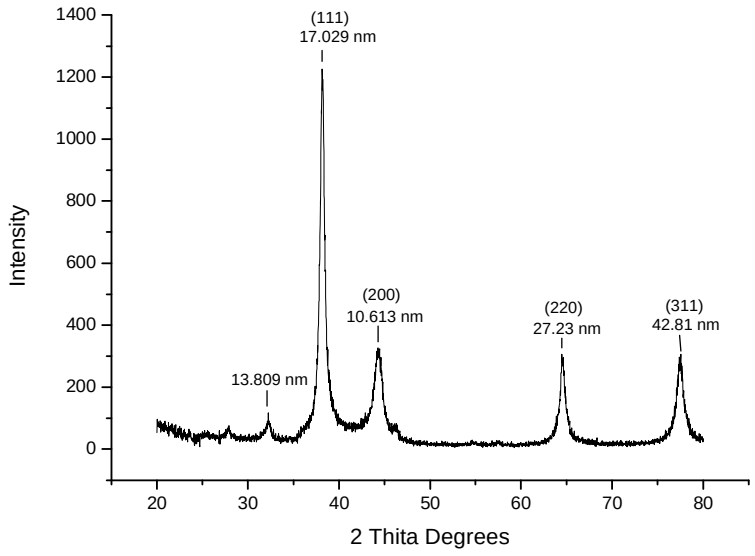


Figure 4. XRD spectrum of biosynthesized CR AgNPs

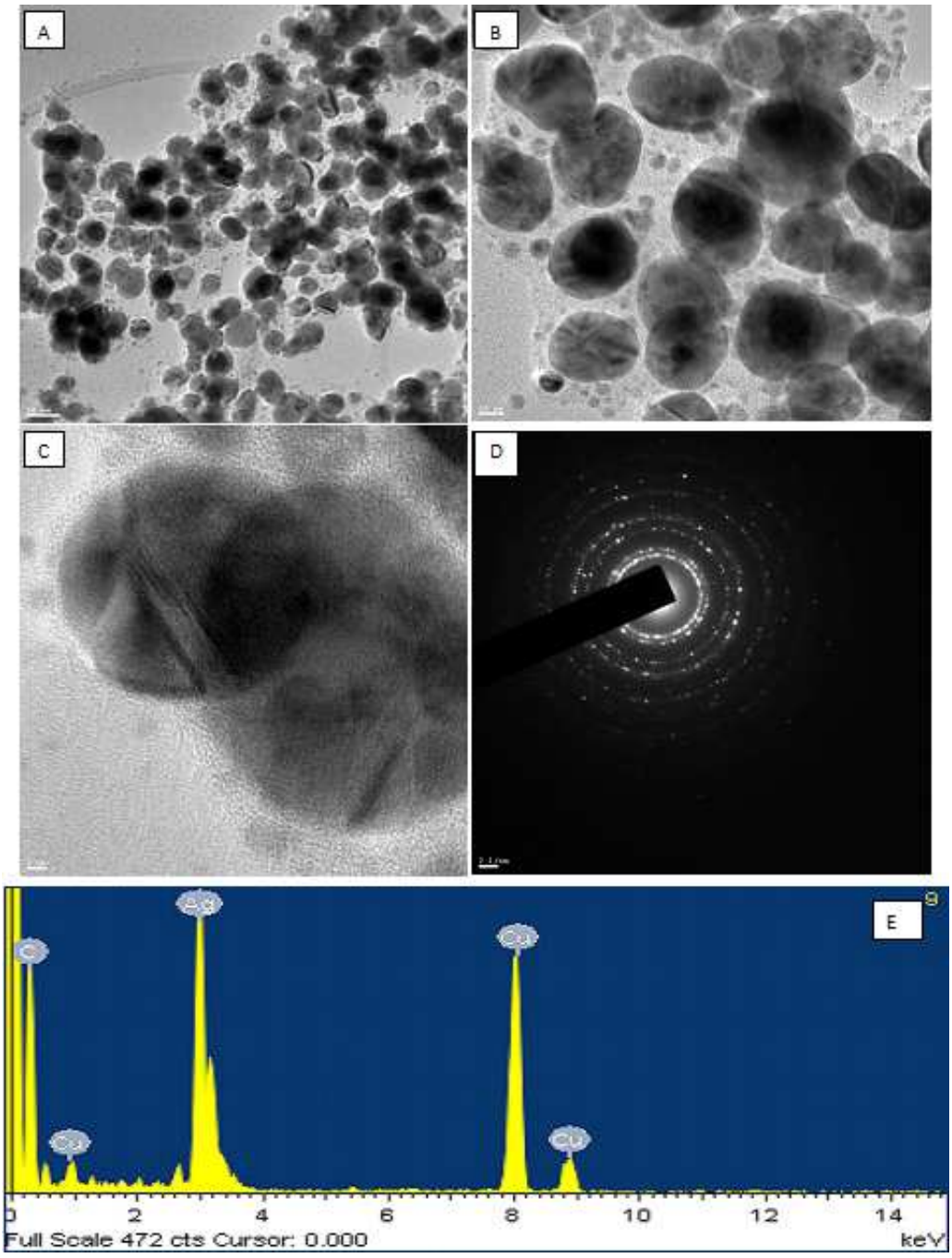


Figure 5. TEM images (A, B, C) of Ag nanoparticles in low and high magnification, (D) SAED patterns of the silver nanoparticles, (E) EDX spectrum showed higher percentage of silver signal

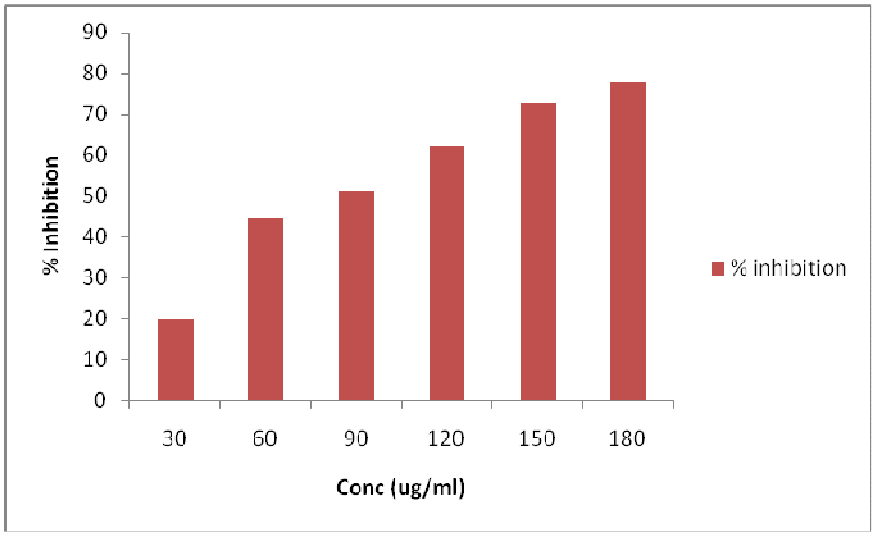


Figure 6. ABTS radical scavenging activity of silver nanoparticles