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By

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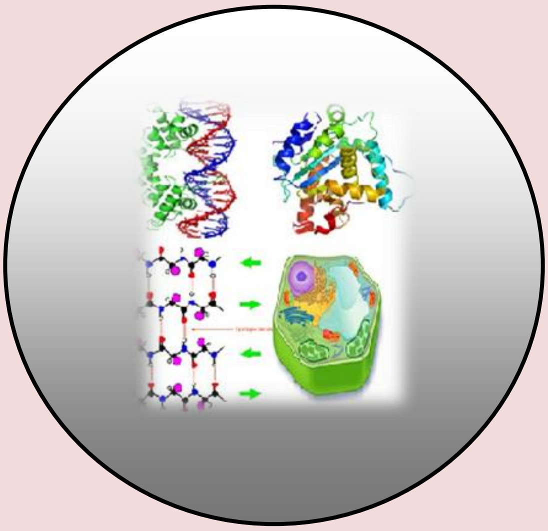
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 36 (1), Part C, 2019 Pages No. 107-115



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 36, No. 1, Part C: 107-115, 2019
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 36/01/7799/2019

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

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RESEARCH PAPER

Received: 04/04/2019 Revised: 21/04/2019 Accepted: 22/04/2019

Characterization of *Cuscuta reflexa* Silver Nanoparticle and its Amelioration effect on Waste Water Treatment

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ABSTRACT
In the recent years there has been a very rapid growth of world population, increasing exponentially the demand for fresh water. But, the existing fresh water bodies in most parts of the world are believed to be highly contaminated. Nanotechnology is now providing new avenues to alleviate this problem by treatment of waste water and polluted water with nanoparticles. This study focuses on investigation of the reduction of toxicants in the waste water by using Cuscuta reflexa silver nanoparticle. The study proves that the plant extract has antibacterial effects as well as a role in wastewater effluent treatment.
Keywords: Cuscuta reflexa, Silver Nanoparticle, Antibacterial Activity and Waste Water Treatment.

INTRODUCTION
The enduring increase of the global need for the water has been closely related to the growth of World population and the climatic changes where, constant growth of the World's population is estimated to be approximately doubled from 3.4 billion in 2009 to 6.3 billion people in 2050, which in turn predicts that the need for the growth of agricultural production of it to be 70%, by 2050 (Bruinsma, 2009). Ultimately, the demand for fresh water is also increasing exponentially. At present, 64 billion cubic meters of fresh water are progressively consumed each year throughout the World (World Water Development Report 4, 2014). In both the developing and the industrialized countries, increased consumption of contaminants like micro pollutants are believed to enter the water bodies. The remodeling of highly advanced techniques in the nanotechnology compared to that of the traditional processes in engineering offers new opportunities for the development of advanced water and wastewater treatment processes. Nanotechnology being one of the leading growing technologies has the art of manipulating matter at the scale of 1-100 nm and recently it also offers the potential of novel nanomaterials for the management of wastewater, surface water and groundwater contaminated with the toxic organic, inorganic solutes, metal ions and toxic effluents. Nanoparticles have unique activity towards the intractable contaminants and currently many nanomaterials are under active research and development (Bruinsma, 2009).

Nanosilver as a nanomaterial has been used in the various applications, such as photo development for the past several years, and has been registered with the Environmental Protection Agency as one of the key component for its use in swimming pool algaecides since 1900s and drinking water filters since the 1970s (Gamble, 1972). Recent studies show that the nanomaterials as nanosilvers exhibit a strong and broad-spectrum antimicrobial activity and has hardly shown any harmful effects over the humans. Hence, it is already applied as one of the point-of-use water disinfection systems and anti-biofouling surfaces (Tognolini et al., 2006). In the present study it has been shown that the use of phyto-based nanomaterials using *Cuscuta reflexa.* Roxb, in recent advances to treat tap water treatment and wastewater effluent removal processes. Besides this the study also shows promising technological enhancements and limitations of nanotechnology for wastewater treatment applications, health risks and advantages that are reported.

C. reflexa is a leafless and rootless perennial parasitic twining herb of Convolvulaceae family, and it is commonly known as the Akashvalli or Dodder. The plant is widely distributed worldwide and in India about 6 species are found presently (Asthana et al., 2009). The plant lacks chlorophyll making it incapable to prepare its own food by photosynthesis (Stanely et al., 1999). Hence, it grows on thorny or normal shrubs and it completely covers various perennial bushes and trees (Oyanedel-Craver et al., 2008). It is observed to spread from one host to another with special branching organs called haustorium. The haustorium penetrates on the host and connects to the xylem in order to connect to the host phloem and absorb both water and elaborated food, such as sugar and amino acids (Bielefeldt et al., 2009). The plant's germination of seeds can occur without the help of the host and for this it has to penetrate and reach a green plant rapidly. The *C. reflexa* has been investigated for its antitumor (van Halem et al., 2009), antimicrobial (Getman, 2011), hepatoprotective (Biosafe, 2011), anticonvulsant (Kimble, 2012), antioxidant (Zhang and Oyanedel-Craver, 2012), induced alopecia (Vigeant et al., 2002) activities and has been screened for various chemical constituents isolated such as cuscutin, amarbelin, beta-sterol, stigmasterol, myricetin, qurecetin, cuscutamine, luteolin, bergenin (Cumberland and Lead, 2009). The flowers of the plant are small and bell shaped white in colour seen with yellow filaments. Fruits and seeds of the plant are used in producing traditional medicines for the treatment of headache, labour pain, bone fracture, fever, and rheumatism (Song et al., 2011).

Use of Nanoparticles in waste water treatment

Nano-based structural materials are of different types mainly such as carbon nanotubes, silver-impregnated cyclodextrinnano-composites, magnetic nanoparticle, nanostructure iron zeolite, carbon-iron nanoparticles, photocatalytictitania nanoparticles, nanofiltration membranes and functionalized silica nanoparticles and these are the effectively employed structural Nanoparticles used in water treatment to remove contaminants such as heavy metals, sediments, chemical effluents, charged particles, bacteria and other pathogens. In particular, Silver Nanoparticles (AgNPs) are the most commonly used nanoparticles seen in a wide range of applications, including solar energy absorption and chemical catalysis and disinfection (Fontes et al., 1991; Arora et al., 2008; Beer et al., 1999). Recently implemented nanoparticles as consumable products contain AgNPs account for more than 25% of the 1,015 nanotechnology-based products that are available in the market (Bloem et al., 2009). Previous studies on AgNPs show that they present large surface areas per volume ratio and high reactivity compared to that of bulk solids. This characteristic gives AgNP the ability of possessing antimicrobial properties. Three possible antimicrobial mechanisms of AgNP are thought to be a) the AgNP damage cell membrane and intracellular components (Cachafeiro et al., 2007; Chernusova and Epple, 2013), b) silver ions released from AgNP can be absorbed into the cell wall and cause lysis and death (Grosse et al., 2003; Khan et al., 2011)and c) reactive oxygen species (ROS) can be formed in AgNP solution (Haase et al., 2012a; Kermanizadeh et al., 2013; Kim et al., 2011). Some evidence shows that the disinfection effectiveness of AgNP is size dependent (Chindris, 2010), and that the process of aggregation reduces their surface area, reducing the cell-particle interaction, membrane penetration, and the rate of silver ion release (Wijnhoven et al., 2009). Thus, this study aims to investigate the reduction of environmental toxicants found in the nano-based *C. reflexa* silver nanopartricle for further investigation.

MATERIALS AND METHODS

Plant collection

The *C. reflexa* plant leaves were collected fresh from the foothills of the Nilgiri region. The collected plant leaves said above were shade dried and powdered to contribute to 57g of the plant extracts as powder.

Green synthesis of Silver Nanoparticles

The plant *C. reflexa* was chosen selectively and analyzed for its use full morphologies and the leaves were collected and were used for the silver nanoparticle synthesis respectively, which was prepared using the traditional biological or the green synthesis protocol (Marambio-Jones and Hoek, 2010). 99ml of the 1% silver nitrate ($AgNO_3$) solution was added with the 1ml of respective pre dissolved and filtered plant extracts. The observations of color change from a transparent colorless solution to deep a brownish yellow color formation for the confirmation of the plant - silver nanoparticle synthesis completion were also made and were confirmed using the spectra analysis at 400 - 570nm in the UV-spectrophotometer system using the samples collected at various time intervals during the synthesis procedure.

Characterization of Nanoparticles

The synthesized nanoparticles of the each individual specimen plants prepared were checked for presence of various functional groups using the Fourier Transform - Infrared Radiation (FT-IR) characterization tool and the size, morphology and the silver nanoparticle confirmations were made by the Field Emission-Scanning Electron Microscope (FE-SEM), Energy Dispersive Analysis of X-rays (EDAX) characterization tools.

Antibiotic Susceptibility Test

The Antibiotic Susceptibility Test (AST) was carried out for the in the current study the biologically synthesized *C. reflexa.* AgNPs were checked for their antibacterial activity using the antibiotic sensitivity test for cultures of *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively using the Kirby-Bauer disk diffusion susceptibility test protocol (Lu et al., 2008).

Waste water treatment analysis

Organic matter is mainly composed of compounds such as proteins, carbohydrates, and fats, biodegradable organics that are measured in terms of biochemical oxygen demand (BOD) and chemical oxygen demand (COD). If there is presence of any untreated discharge to the environment, its biological stabilization can cause the depletion of natural oxygen sources and the quality of fresh water in available sources leading to fresh water contamination (Elechiguerra et al., 2005). The collected tap water and waste water was taken in each separate 500 ml in round-bottom flask. The pre-prepared green synthesized $AgNO_3$ leaf extract was added into the 500 ml beakers containing the tap water and waste water with a concentration rate of 5 ml and the tap water and the waste water respectively with the concentrations approximately set to around 10 ml in the round bottom flask. A set of three experiments were conducted for calculating the approximate results. The flasks were initially stirred with the nanoparticle pellets and mixed for around 15 minutes and then heated up to 45 minutes using water bath at 70° C. After heating, the samples were drawn at regular intervals of every 5 minutes and were checked for pH, TDS, total hardness, BOD, COD as per APHA (American Public Health Association) standards.

BOD or biodegradable soluble Chemical Oxygen Demand (bsCOD)

The bsCOD of the effluent was calculated the following formula,

$$S = \frac{K_S\left(1 + k_d(SRT)\right)}{(SRT)\left(Yk - k_d\right) - 1}$$

where, S = effluent soluble substrate concentration (bsCOD), SRT = Sedimentation Retention Time, Ks = half-velocity constant, kd = endogenous decay coefficient, Y = biomass yield, k = maximum specific soluble substrate utilization rate, fd = fraction of biomass that remains as cell debris

Similarly, the TDS is calculated by the following formula:

$$\text{mg Dissolved Solids/L} = \frac{(A-B)}{\text{mL Sample}} \times 1000$$

Whereas, the Total hardness of the samples were calculated using the formula,

Water hardness (mg/L) = Ca (mg/L) × 2.497 + Mg (mg/L) × 4.118

RESULTS AND DISCUSSION

Silver and AgNPs show high range of antibacterial properties against a wide range of both Gram-negative (e.g. *Acinetobacter, Escherichia, Pseudomonas, Salmonella* and *Vibrio*) and Gram-positive bacteria (e.g. *Bacillus, Clostridium, Enterococcus, Listeria, Staphylococcus and Streptococcus*) (Sun et al., 2010). Recently many research works show that the AgNPs also demonstrate the wide range of antifungal activity against various fungal species, such as *Aspergillus niger, Candida albicans and Saccharomyces cerevisia* (De Gusemme et al., 2011). In addition, a number of studies have suggested that they also posses biocidal action of AgNPs against hepatitis B virus (Feng et al., 2000), HIV-1 (Sondi and Salopek-Sondi, 2004), syncital virus (Hewson et al., 2010) and murine norovirus (Mainzen Prince et al., 1999).

UV-Visible spectra

The synthesized *C. reflexa* silver nanoparticles retrieved at various time intervals such as 5min, 10min, 15min, 20min and 25min from light to dark brown coloration show the results given in the Table 1 and the spectra shown in the Figure 1 confirms the presence of AgNPs in the synthesized *C. reflexa* silver nanoparticle mixture.

Table 1. UV-Visible spectra.

Time (minutes)	Color change seen	Optical density value at 420 nm
5	Pale yellowish green	0.456
10	↓	0.578
15		1.753
20		2.098
25	Deep yellowish brown	2.789

FT-IR spectral analysis

The pellets retrieved after the centrifugation at 5000 rpm for 30 minutes of green synthesized AgNPs plant extracts were dried and analyzed for the FT-IR spectra and the Figure 2 depicts the clear picture of the results. It is confirmed that to identify the bio-molecules for reduction of the metal nanoparticles in the *C. reflexa* silver nanoparticles show the band at 3367 cm^{-1} of O-H stretch and H-bonds, 2926 cm^{-1} of alcohols and phenols with shifts from 3460 cm^{-1} and 3168 cm^{-1} seen for the crude plant extract in line with Premasudha *et al.* studies (Paramasivam Premasudha et al., 2015). These results suggest that the molecules perform dual functions of formation and stabilization of silver nanoparticles.

FE-SEM and EDAX Analysis

The *C. reflexa* silver nanoparticles were checked for their structural confirmations to be 108 nm using the FE-SEM analysis and are shown in the Figure 3 and the EDAX of the sample was also characterized and is given in the Figure 4 respectively. Recently the studies given by Dakeshwar on the green synthesis of silver nanoparticles from *C. reflexa* plant extract also show the similar results of sphere shaped nanoparticle in SEM analysis (Dakeshwar, 2018).

Antibiotic Susceptibility Test

Recent studies have shown that the performance of AgNP (<30nm) impregnated fibre glass during immersion and during filtration (Selvam et al., 2017). For the immersion test, a silver impregnated mat (1% Ag by weight) was added to a 100ml *E. coli* suspension (106 cfu/ml). After an hour of immersion, *E. coli* could not be detected in the suspension. Using an *E. coli* concentration of 1012 cfu/ml, the AgNP fiber glass mat (1.8% Ag by weight) resulted in a 7 log reduction in concentration in five minutes. Antibacterial filters (5% Ag by weight) were fabricated and a bacterial solution (106 cfu/ml *E. coli*) was pumped through the filter at a flow rate of 20 ml/minute. *E. coli* were not found in the treated water.

Another notable study conducted as a comparative analysis of disinfectants for use in household water purifiers and considered both silver ions and AgNP, using a batch disinfection test of ground water spiked with *E. coli* (Patil et al., 2012). As with Ag ions, AgNP (synthesized from $AgNO_3$ using citrate as a reducing agent) required a 3 hour contact time, however, a lower minimum concentration of active disinfectant was required (1mg/l).

In the current study the antibiotic sensitive test for the cultures of *E. coli, P. aeruginosa* and *S. aureus* plates show wide range of zone of inhibition for the added biologically synthesized *C. reflexa* AgNPs as shown in the Table 2

Table 2. Antibiotic sensitivity test.

Plate specification	*E. coli*	*P. aeruginosa*	*S. aureus*
Control Plates treated with respective antiobiotic discs	1.3cm	1.57cm	1.27cm
***C. reflexa* AgNPs**	0.97cm	1.2cm	1.2cm

Adsorption analysis for treated waste water product

The pH, TDS, total hardness, BOD, COD were calculated for the effluents of tap water and wastewater before the *C. reflexa* AgNPs treatment and after the treatment for three consecutive days using the following strategies for each as shown in the Figure 5,

The Table 3 shows the complete data of adsorption analysis for all the above parameters for both the tap water and the wastewater effluents taken.

Table 3. Adsorption analysis.

Characteristics checked for	Tap Water			Waste Water			Standard Limits		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
pH	7.9	7.82	7.6	8.54	8.32	8.09	7.5	7.43	7.52
TDS mg/l	3000	2987	2870	4235	4132	3979	2670	2535	2601
BOD mg/l	370	356	384	400	380	378	36	37.5	36.7
COD mg/l	483	479	453	570	567	573	150	147	134.9
Total hardness	618	570	523	729	693	682	310	302	299

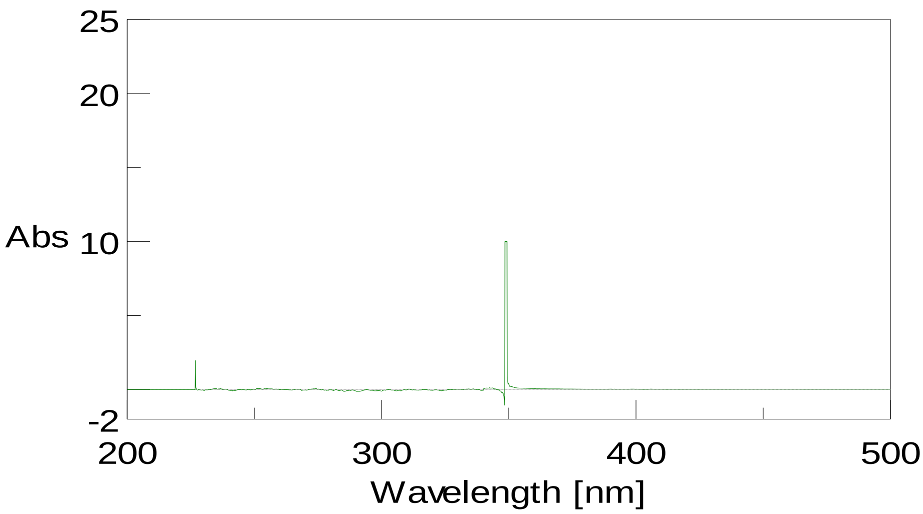

Figure 1. UV visible spectra seen for the C. reflexa AgNPs from 200-500nm.

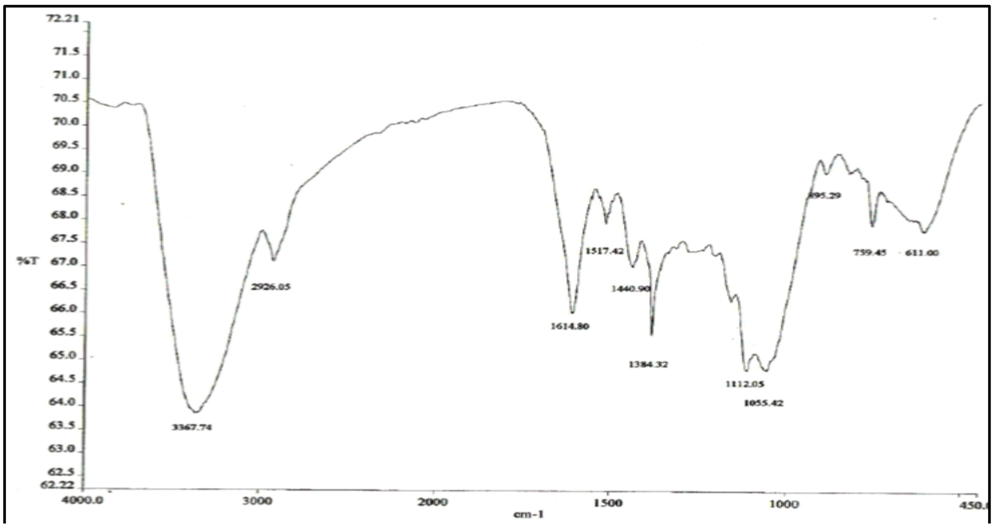

Figure 2. FTIR spectrum showing various functional group presence through spectrum taken from 4000 - 450 nm.

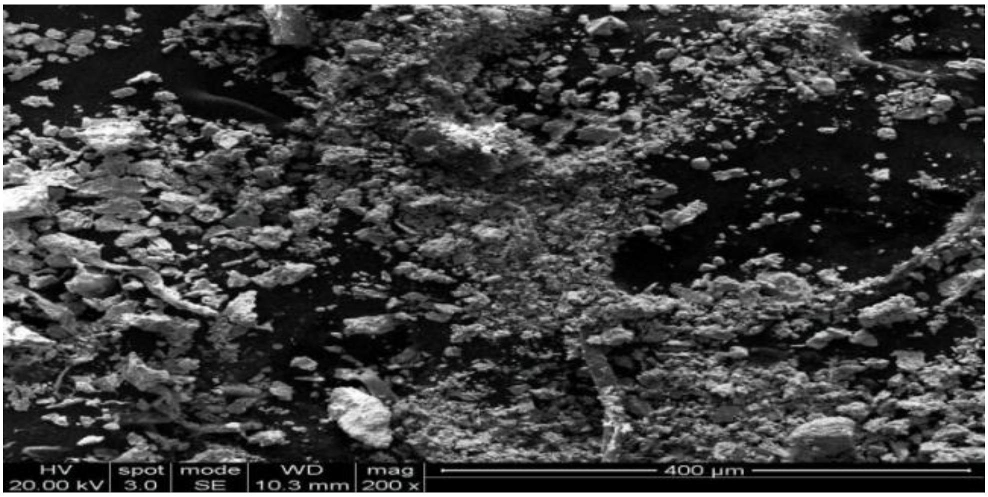

Figure 3. ***C. reflexa*** **AgNPs seen under FE-SEM showing the presence of spherical synthesized AgNPs.**

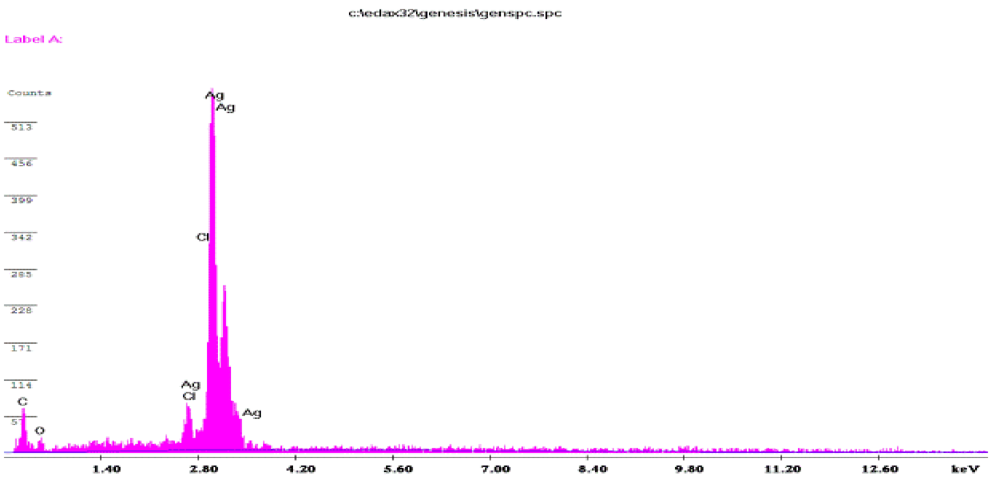

Figure 4. Image showing chemical compounds present in the synthesized AgNPs as seen by EDAX.

Figure 5 Image showing treatment of (a) Tap water (b) Waste water with *C. reflexa* AgNPs.

CONCLUSION

The chronic effluent exposure of the wastewater contaminants shows a wide range of hazardous effects over the environment. The present study depicts that the *C. reflexa* AgNPs show a well-built antibacterial and effluent degradation capacity compared to that of the standard carried out by the control AgNPs. This proves that the plant extract apart from the fact of showing an evident antibacterial effects from the past studies, it also has a positive effect of wastewater effluent treatment from the current study and its data interpretations.

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