Green Synthesis of Silver Nanoparticles Using Cressa Cretica Leaf Extract and its Antibacterial Efficacy

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Abstract: Nanomaterials are widely used in a variety of fields. Nanomaterials are commonly synthesized using physical, chemical and biological methods. The biological green synthesis methods are rapid, cost effective and eco-friendly. The present investigation deals with biosynthesis of silver nanoparticles from silver nitrate using cressa cretica leaf extract. The biosynthesized silver nanoparticles were characterized by UV-Visible spectrophotometer, X-Ray Diffraction, FTIR spectra, EDX and Scanning Electron Microscopy. The antibacterial activity of biosynthesized silver nanoparticles was investigated by diffusion disk method. It is suggested that the biologically synthesized silver nanoparticles are more effective against various disease causing pathogens.

Key words: silver nanoparticles, cressa cretica, UV-visible spectra, XRD, antibacterial activity

I. INTRODUCTION

Nanotechnologies are among the fastest growing areas of scientific research and have important applications in a wide variety of fields. Nanomaterials are commonly synthesized using physical, chemical and biological methods. The biological green synthesis methods are rapid, cost effective and eco-friendly. The present investigation deals with biosynthesis of silver nanoparticles from silver nitrate using cressa cretica leaf extract. The biosynthesized silver nanoparticles were characterized by UV-Visible spectrophotometer, X-Ray Diffraction, FTIR spectra, EDX and Scanning Electron Microscopy. The antibacterial activity of biosynthesized silver nanoparticles was investigated by diffusion disk method. It is suggested that the biologically synthesized silver nanoparticles are more effective against various disease causing pathogens.

Plants have flavonoids, alkaloids and polyphenolic compounds which may reduce the silver ions to silver nanoparticles and acts as capping and stabilizing agent [9].

The silver nanoparticles are highly toxic to several pathogenic organisms and hence play a vital role in treatment of many diseases [10,11]. Silver has long been recognized as having an inhibitory effect towards many bacterial strains and microorganisms [12]. Antibacterial activity of the silver containing materials is used in medicine to reduce infections in burn treatment [13] and arthroplasty [14], as well as to prevent bacteria colonization on prostheses [15], catheters [16], vascular grafts, dental materials [17], stainless steel materials [18], and human skin [19]. Silver nanoparticles also exhibit a potent cytoprotective activity towards HIV-infected cells [20].

Cressa cretica (Linn) belonging to family Convolvulaceae, commonly known as Rudravanti is a erect, small, dwarf shrub, [21] usually grows in sandy or muddy saline habitats [22]. Cressa cretica is a remarkable salt tolerant plant, common in coastal areas [23] usually occurring in mono specific stands along the landward edge of marshes [24]. This plant is distributed throughout India, Timor, and Australia (Western Australia, Northern Territory, Southern Australia, Queensland, New South Wales, Victoria). The plant is used as antibilious, antitubercular, expectorant [25]-[26], antihelminthic, stomachic, tonic and aphrodisiac purposes, and enriches the blood and is useful in treating constipation, leprosy, asthma, urinary discharges, and as an appetizer [27]. Dry leaves of Cressa cretica crushed with sugar are used as emetic in Sudan [28].

Fig:1 Cressa cretica
Vernacular names[29]-[32]

Hindi – Rudravanti
Tamil – Uppu Marikkozhundu
Telugu – Uppusanaaga
Sanskrit – Rudanti
Bengali – Rudravanti

Cressa cretica leaves contain bioactive constituents such as carbohydrates, flavonoids, phytosterols, terpenes, tannins, glycosides, fixed oil and sugars[33,34]

Based on the importance of green synthesis of silver nanoparticles from leaf extract, the present study was carried out to synthesize and characterize the silver nanoparticles using Cressa cretica (Linn) leaf extract.

II. MATERIALS AND METHODS:

2.1. Collection of plant samples:

Cressa cretica plants were collected from Tuticorin District, Tamilnadu, India. The plant leaves were thoroughly washed thrice with tap water and then with double distilled water to remove dust particles, air-dried for a week under shade at room temperature, finely cut, milled into a fine powder and was stored in airtight containers for later analysis.

2.2. Preparation of aqueous plant leaf extracts:

15 gm of the leaf powder was mixed well with 100 ml of double-distilled water and boiled at 60 °C for 30 min. After boiling, the extract was filtered through Whatman No.1 filter paper. The supernatant was collected and stored at 4 °C for further nanoparticles process[35].

2.3. Synthesis of silver nanoparticles:

1 mM aqueous solution of Silver nitrate (AgNO₃) was prepared and used for the synthesis of silver nanoparticles. 1 ml of Cressa cretica leaf extract was added into 100 ml of aqueous solution of 1 mM silver nitrate for reduction of Ag⁺ ions and kept at room temperature for 24 hours[36]. Formation of reddish brown colour confirmed the silver nanoparticles.

III. CHARACTERIZATION STUDIES:

The green synthesized silver nanoparticles were characterized by the following methods:

3.1. Visual Observation:

A change of colour from pale yellow to reddish brown was observed in the solution after visible irradiation.

3.2. UV Spectrophotometric analysis:

The formations of leaf extract mediated silver nanoparticles were confirmed by the spectral analysis. The UV spectra of the biosynthesized silver nanoparticles were recorded using shimadzu UV-1800 Spectrophotometer by continuous scanning from 200nm to 900nm and distilled water was used as the reference for the baseline correction.

3.3. Fourier Transform Infra Red Spectroscopy Analysis:

The functional groups in the biosynthesized Ag NPS solution were analyzed by FTIR spectroscopy. These measurements were carried using a perkin Elmer spectrum RX I FTIR instrument with a wavelength range of 4000 to 400 nm. The results were compared for shift in functional peaks.

3.4. Field Emission Scanning Electron Microscopy:

FESEM was used to characterize the mean particle size, morphology of the AgNPs. A small drop of biosynthesized Ag NPS solution was placed on glass slide and allowed to dry. The samples were analyzed by using FEI Quanta 200 FEG machine at a low vacuum in the range 10-20 kV

3.5. Energy Dispersive X-ray Analysis:

The elemental composition of the synthesized nanoparticles was analyzed with energy dispersive spectroscopy coupled to scanning electron microscope.

3.6. XRD analysis:

The silver nanoparticles solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 20 min. The purified Ag nanoparticles are dried. The structure and composition of Ag nanoparticles were studied by XRD (XPERT-PRO Machine). The data was collected in the 2θ range. The crystalline domain size was calculated from the width of XRD peaks using Scherrer’s equation.

Dabje- Scherrer’s equation, \( D = K \lambda / \beta \cos \Theta \);

Where, \( D \) = average crystalline domain size; \( \beta \) is the Full Width at Half Maximum (FWHM), \( K=0.94 \), \( \lambda \) = wave length of X ray, and \( \Theta \) is the diffraction angle.

3.7. Antibacterial activity:

The antimicrobial activity of AgNPs was estimated against pathogenic bacteria such as Enterococcus faecalis, Staphylococcus aureus (gram- positive bacteria), Pseudomonas aeruginosa, E. coli (gram-negative bacteria) by disc diffusion method[37]. The bacterial cultures were grown in Brain Heart Infusion liquid medium at 37 °C. After 12 hrs of growth, each microorganism, at a concentration of 1 x 10⁶ cells/mL equivalent to 0.5 Mc Farland Standard was spread on the surface of Mueller-Hinton agar plates. The dilutions were made in sterile low glucose Nutrient broth. Test pathogens were spread on the test plates- Muller Hinton agar for bacteria. The synthesized Ag nanoparticles and positive control were loaded onto 6 mm diameter sterile discs and placed in the plates respectively. After 24hrs of incubation, the zone of inhibition (mm in diameter) was measured and taken as the activity against the test pathogen.[38]
IV. RESULTS AND DISCUSSION:

4.1. UV-Visible Spectroscopy analysis:

The nanoparticles were primarily characterized by UV-Vis spectroscopy for the analysis of nano particles.

![Colour change indicating formation of Ag NPs](image1)

Fig-2: Colour change indicating formation of Ag NPs

Fig-2: solution of (A) Cressa cretica leaf extract (B)1m M AgNO₃ Solution without leaf extract (c) 1m M AgNO₃ Solution with leaf extract after 5 mins (D) 1m M AgNO₃ Solution with leaf extract after 24hrs

The absorption spectrum of Ag NPs from cressa cretica leaf extract is shown in fig.3. A well defined peak at 444.5nm exhibited by the nano metallic Ag particles and broadening of peak indicated that the particles are polydispersed\[39]-[40]. The colour change from the colourless to reddish brown due to the excitation of free electrons in the nanoparticles[41]. It confirmed the successful synthesis of Ag NPs.

4.2. FT-IR analysis:

Fig.4. shows the FT-IR spectrum of synthesized Ag NPS from leaf extract of cressa cretica. FT-IR measurement was carried out to identify the possible bio molecules responsible for capping and reducing agent for the AgNPs synthesized by cressa cretica leaf extract. The peak at 3437.43 cm\(^{-1}\) corresponds to O-H stretching of phenols and alcohols and N-H stretching of amines. The peak at 2360.37 cm\(^{-1}\) corresponds to C≡N stretching of nitriles and C≡C stretching of alkynes, the peak at 2068.86 cm\(^{-1}\) corresponds to CO\(_2\) molecule ,the peak at 1643.80 cm\(^{-1}\) corresponds to C=O stretching of aromatic ester and enolic ketones , the peak at 1312.60 cm\(^{-1}\) corresponds to C-O stretching of ester and phenols and C-N stretching of amines, peaks at 671.40 cm\(^{-1}\) corresponds to C-Cl stretching of aryl halides. These bio molecules reduced Ag\(^+\) to Ag as well as stabilizing Ag NPs.

![FT-IR Spectra of silver nanoparticles synthesized from cressa cretica leaf extract](image2)

Fig -3: UV Spectral analysis of silver nanoparticles synthesized from cressa cretica leaf extract

Fig -4 : FT-IR Spectra of silver nanoparticles synthesized from cressa cretica leaf extract
4.3. XRD Analysis:

Fig. 5: XRD patterns of silver nanoparticles using cressa cretica leaf extract

The green synthesized silver nanoparticles are highly crystalline with diffraction peaks could be obviously assigned to the face-centered cubic phase of metallic silver. Fig. 5 shows main characteristic diffraction peaks for Ag observed at 20 values of 38.03°, 44.46°, 64.65° and 77.32° are indexed to the (100), (200), (220) and (311) reflections of the fcc structure of metallic silver respectively. The average grain size of the silver nanoparticles formed in the bio reduction process was determined using Scherer's formula.

4.4. SEM and EDX analysis:

Fig. 6: SEM image of Ag nanoparticles using cressa cretica leaf extract

Fig. 6 shows the morphology investigated using scanning electron microscopy (SEM). This picture reveals that the green synthesized silver NPs are approximately rod like structure and the particles are slightly agglomerated and the dimensions of nanorods are between 50–200 nm in width and few microns in length. The results of energy-dispersive spectroscopy (EDX) analysis are shown in Fig. 7. It is confirmed that the significant presence of elemental silver, which indicates bio reduction of silver ion to elemental silver. The strong signal in the silver region was observed at 3 keV for silver nanoparticles due to the Surface Plasmon Resonance [42]-[43].

Fig. 7: EDX image of Ag nanoparticles using cressa cretica leaf extract
4.5. Anti bacterial Activity:

Silver is well known as one of the most universal antibacterial substances. Antibacterial activity is investigated against Enterococcus faecalis, Staphylococcus aureus (gram-positive), Pseudomonas aeruginosa, E. coli (gram-negative) by disc diffusion method are shown in Fig. 8.[44]. The diameter of inhibition zones (mm) around each well with silver nanoparticles solution are represented in Table 1. The table.1 shows that the zone of inhibition (ZOI) was increased when increasing the concentration of Silver nanoparticles[45]. The maximum antibacterial activity in 500µg/ml concentration of the synthesized Ag nanoparticles was 14 mm for pseudomonas aeruginosa, 11mm for E. coli, 10 mm for staphylococcus aureus and 14 mm for Enterococcus faecalis receptively. The silver nanoparticles synthesized by cressa cretica leaf extract are found to have highest antibacterial activity against pseudomonas aeruginosa (14 mm) and Enterococcus faecalis (14 mm) respectively and the lesser antimicrobial activity of silver nanoparticles is found against staphylococcus aureus(10 mm). The antibacterial activity of Ag NPS studies confirmed that the Ag NPs synthesized by leaf extract of cressa cretica exhibited good antibacterial potential against gram-positive and gram-negative bacterial strains.

Table : 1 Zone inhibition of Silver nanoparticles

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<th>Serial. No</th>
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Fig. 8 : Antibacterial activity of silver nanoparticles synthesized by cretica leaf extract against human pathogens

V. CONCLUSION :

The present study concluded that the leaf cressa cretica can be used as an excellent source for synthesizing the silver nanoparticles. The primary confirmatory for the silver nanoparticles was colour changes and Uv-Vis absorption spectra of silver nanoparticles forming peak at 444.5 nm. The SEM image confirmed that the green synthesized silver nanoparticles are rod in shape. The green synthesized nanoparticles have more effective antibacterial activity to the pathogens. The present study emphasizes the use of medicinal plants for synthesis of silver nanoparticles with potent antibacterial effect. The Applications of such eco-friendly nanoparticles in
bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials).

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VII. REFERENCES: