Bioavailability of Green Silver Nanoparticles using *Belosynapsis kewensis*

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ABSTRACT *Silver nanoparticles (AgNPs) were synthesized using Belosynapsis kewensis (B. kewensis) leaf extract and the extract was acted as a reducing, stabilizing agent. The formation of AgNPs was observed by Ultraviolet-visible spectroscopy (UV-vis spectroscopy) where surface plasmon resonance (SPR) occurring at 420nm. The Fourier Transform Infra Red spectroscopy (FTIR) spectrum showed prominent peaks (3442, 2898, 1602, 1527 and 1445cm^{-1}) in the region of 4000 – 400cm^{-1}. The X-Ray Diffraction (XRD) peaks showed at 38.02°, 43.99°, 64.48° and 77.31° which were corresponding to (111), (200), (220) and (311) planes for face centered cubic (FCC) structure with an average size of particles was found to be 28.46nm. The Field Emission Scanning Electron Microscopy (FESEM) image showed the AgNPs with an average size ranged from 10 to 30nm. The Energy Dispersive X-Ray Spectroscopy (EDX) analysis proved that the required phase of silver (Ag) is present in the sample. The green synthesized AgNPs of B.kewensis expressed excellent ant0ioxidant (DPPH) and antibacterial activity against human pathogens like P.aeruginosa, S.aureus and B.subtilis.*

Keywords: *Belosynapsis kewensis, Silver Nanoparticles, Green synthesis, antibacterial activity, Surface Plasmon Resonance*

1.Introduction

Nanotechnology is the research and technology development at the atomic, molecular or macromolecular levels, in the length scale of approximately 1 - 100 nanometer range. It is the surface to volume ratio in any nanostructure which makes this technology very attractive for numerous applications. Green synthesis of NPs using biological resources like plants and micro-organisms provide the range of advantages such as eco-friendly, cost effect, non-hazardous and non-toxic [1]; [2]. The development of green approach for the synthesis of NPs is extending into an important branch of nanotechnology. Since, the ancient period metal NPs have used as novel source of an antioxidant and antibacterial agent. Silver has been recognized as having inhibitory effect on microbes present in medical and industrial process [3]. The biologically synthesized AgNPs were found highly toxic against different multi-drug resistant human pathogens [4]. The advantages in biological synthesis of AgNPs are that they are easily available, safe to handle and consists wide range of metabolites, which can aid in the ionic reduction [5]. Antioxidants are capable to deactivate the free radicals, and can cause extensive damage to cells as a result of imbalance between the generations of Reactive Oxygen Species (ROS) [6]. The antibacterial activity of AgNPs has been authenticated in the recent years by Michał *et al*., [7]; Mudasir *et al*., [8]; Amit Kumar *et al*., [9]. *B.kewensis* is a member of commenlinaceae with high medicinal importance. The antioxidant potential of this plant has been reported by Premkumar *et al*., [10]. Hence the aim of the present study is to develop a novel approach for the biosynthesis of AgNPs using *B.kewensis* leaf extracts at room temperature, characterization and its applications in antioxidant and antimicrobial activities. The antibacterial activity of AgNPs was investigated against different human pathogenic bacterial strains.

2. Materials and Methods

2.1 Chemicals and Plant material Collection

Silver nitrate ($AgNO_3$) and dimethyl sulfoxide (DMSO) were purchased from Merck, India. The glasswares were washed thoroughly with acid and followed by rinsing with Millipore-Milli-Q water. The

fresh and mature leaves of *B.kewensis* were collected from Manjolai hills, Thinelveli district, Tamilnadu, India.

2.2 Biosynthesis of AgNPs

The collected leaves were washed thoroughly with running water followed by de-ionized water. 10g of cleaned leaves were boiled at 80°C for 10min in 100ml with deionised water and crude extract was filtered by Whatman No.1 filter paper finally the extract was stored at 4°C for further uses [11]. The obtained extract of *B.kewensis* was used to synthesis of AgNPs as reducing as well as a stabilizing agent of silver ions. To facilitate the synthesis of AgNPs 10ml of *B.kewensis* leaf extract was mixed with 90ml of 1mM $AgNO_3$ solution. This reaction mixture was kept at room temperature. Later, the color change was observed to designate the formation of colloidal AgNPs.

2.3 Characterization of AgNPs

The biosynthesis of AgNPs was confirmed by UV-vis spectroscopy (Hitatchi-2001) in a wavelength ranges between 200-800nm at 1nm resolution. Synthesized AgNPs was diluted with deionized water and centrifuged at 10000rpm for 15 min. The residue was dispersed with deionized water twice to remove the biological impurities. The purified residue was dried in oven at 70°C for overnight. The AgNPs was used for FTIR analysis to identify the functional groups on a Perkin-Elmer spectrum instrument at a resolution of $4cm^{-1}$ in the transmission mode of 4000-400cm^{-1} in KBr pellets. The particles size and crystalline structure of the green AgNPs were determined by XRD using XPERT-PRO, D-8 at 30Kv, 40mA with CuKα radians at 2θ angle. The crystalline silver nanoparticle was calculated from the width of the XRD peaks and the average size of the nanoparticles can be estimated using the Debye-Scherrer formula ($D=K\lambda/\beta\cos\theta$). The surface morphology of the AgNPs was examined by FESEM (Jeol JSM-6701 FESEM). Thin film of the samples was prepared with aluminium foil by dropping a small amount of the sample onto the copper grid. The reduced silver ions were dried on an aluminium-foil-coated copper grid and EDX analysis of the sample was performed using FESEM (BRUKER-INDIA, FESEM) equipped with an EDX attachment.

2.4 Antioxidant activity of AgNPs

The scavenging activity of DPPH free radical by *B.kewensis* plant leaf aqueous extract and synthesized AgNPs was done according to the method reported by Gyamfi et al [12]. 10µL of 0.1mM DPPH (prepared with ethanol) was added to different concentration of *B.kewensis* aqueous leaf extract and AgNPs. The reaction mixture was shaken and incubated in the dark for 30min. The reduction of the DPPH free radical was measured by reading the absorbance at 517nm. Ascorbic acid was used as the positive control. The lower absorbance of the reaction mixture indicated a higher percentage of scavenging activity. The inhibition ratio was calculated from the following equation:

% inhibition = [(control absorbance–sample absorbance)/control absorbance] X 100

2.5 Antibacterial activity of AgNPs

The antibacterial activity of AgNPs and aqueous leaf extract of *B.kewensis* was assessed by the disc diffusion method against human pathogenic bacterial strains namely *B. subtilis*, *P. aeruginosa* and *S. aureus*. Sterile Whatman no.1 filter paper discs of 5mm diameter were loaded with 20 mg of the sample and placed on nutrient agar plates inoculated with bacterial cultures. The plates were incubated at 37ºC for 24h and the zone of inhibition was measured by using antibiotic zone scale [13]. Standard tetracycline antibiotic discs were used as positive control.

3. Results and discussions

The formation of NPs was done by reduction of Ag^+ ions into AgNPs with exposure of the leaf extract of *B.kewensis* and the formation was seen by the color change. After the incubation period of the suspension was turned from yellow to dark brown in color which indicated the surface plasmon resonance (SPR) [14]; [15]; [16]; [17]. The SPR phenomenon was very sensitive to NP's nature, size and shape, which were formed by their inter particulate distance and the surrounding media [18]; [19]. Fig.1 represents the UV-vis spectrum of green synthesized AgNPs with higher peak level was observed at 420nm and SPR band also exposed at the single peak without any shifting. Logeswari et al., 2015 [20] observed the same absorption band in synthesis of AgNPs using seaweeds. Single peak indicated the synthesized particles were uniform size and shape. So, the formation of AgNPs was attributed by hydrophilic and hydrophobic interaction, which, leads to prevent the particles from aggregation by intermolecular forces [21]. Fig.2 shows the XRD pattern of AgNPs and peak values at 2θ degrees of 38.02°, 43.99°, 64.48° and 77.31° were corresponding to (111), (200), (220) and (311) planes of AgNPs. All the degrees of the peaks were corresponded to a face centered cubic (FCC) crystalline structure. The intense peak 38.02° was represented a high degree of crystallinity [22]. The average size of the AgNPs was estimated by using Scherr's formula,

D=kλ/βcosθ where 'D' is particle diameter size, k is a constant, 'λ' is wavelength of X-ray source (0.1541nm), 'β' is the full width at half maximum (FWHM) and 'θ' is the diffraction angle. Thus, the average size of particles was found to be 28.46nm. FTIR spectrum indicated the clear peaks with (3442, 2898, 1602, 1527 and 1445cm^{-1}) different values (Fig. 3). Above the peak values were corresponded to functional groups like, alcoholic group (0-H stretching Polyphenolic compounds - 3442cm^{-1}), an amine group (N-H stretching – 2898cm^{-1}), a ketone group (bend C=C stretching 1602cm^{-1}), nitro group (N-O stretching 1527cm^{-1}) and amine group (N-H secondary bend - 1445cm^{-1}). The functional groups such as alcohol, amines, ketone and nitro were confirmed their presence in AgNPs. They were denoted as possible biomolecules responsible for stabilizing, capping and reducing agents of the AgNPs [23];[14];[24]. Additionally, amine groups are also responsible for the presence of the some enzymes which, may responsible for the synthesis of metal particles. Further, polyphenols are also proving that having the potential to reduce the silver metals [24]. The presence of AgNPs was confirmed by FESEM, which showed the synthesis of poly-dispersed spherical AgNPs of size that ranged from 10 to 30nm (Fig. 4(a-b) [25]. The Energy Dispersive X-ray (EDX) spectrum analysis indicated strong signal in the silver region and confirmed the formation of silver nanoparticles (Fig.5). Antioxidant activity of green synthesized NPs was assessed by DPPH scavenging assay by using Rutin as positive control. DPPH was a stable antioxidants compound that accepts/receives electrons or hydrogen from AgNPs. Fig.7 showed DPPH assay that free radical inhibition by green synthesized AgNPs as well as *B.kewensis* leaf aqueous extract. The average percentage of inhibition of AgNPs was higher percentage when compared to plant leaf aqueous extract at various concentrations of green synthesized AgNPs. These results are also co-incidence with DPPH scavenging activity by platinum and AgNPs [26]; [27]. The antibacterial activity of aqueous leaf extract and AgNPs was screened against *B. subtilis, P. aeruginosa* and *S. aureus* by disc diffusion method. The culture plates were treated with AgNPs, which exhibit higher antibacterial activity than aqueous leaf extract. The zone of inhibition of AgNPs was found to be 1.9, 1.2 and 1.0cm respective to *P. aeruginosa, S. aureus* and *B. subtilis* as compared to the standard antibiotic tetracycline which produced respective clearance zones of 3.5, 3.3 and 2.8cm at the same concentration of 20mg used in this study. The antibacterial activity of AgNPs with respect their effects on bacteria growth was analyzed against *P. aeruginosa, S. aureus* and *B. subtilis* culture. From the Fig.6 shows the AgNPs higher better antibacterial activity than aqueous leaf extract of *B.kewensis*. The previous reports also indicated that the green synthesized AgNPs exhibited highest antibacterial activity against *E.coli* and *S.aureus* from *Vitex negundo* [28]; papaya fruit derived AgNPs have been showed to display potent antibacterial property against *E. coli* and *P. aeruginosa* and the activity was comparable to standard antibiotics tetracycline and rifamycine [29]. Though silver has been practiced from the ancient time, recent advancement and reinvention of green AgNPs synthesis has became a popular research area of drug discover. The AgNPs exhibit antibacterial susceptible by attaching to the bacterial cell. Since the bacterial plasma membrane is the site of respiratory chain components, energy transducing systems and for active transport of molecules and ions [30]; [31]; [32] any changes in membrane structure would ultimately result in inhibition of bacterial growth.

4. Conclusion

The present study concluded that the green synthesis of AgNPs using *B.kewensis* leaf extract provides simple, rapid, eco-friendly, non-toxic and alternative to physico-chemical methods. This green approach made face centered cubic AgNPs with average size of 28.46nm. The biosynthesized AgNPs exhibited promising antioxidant activity and antibacterial activity. These obtained AgNPs has excellent potential applications in the biomedical applications.

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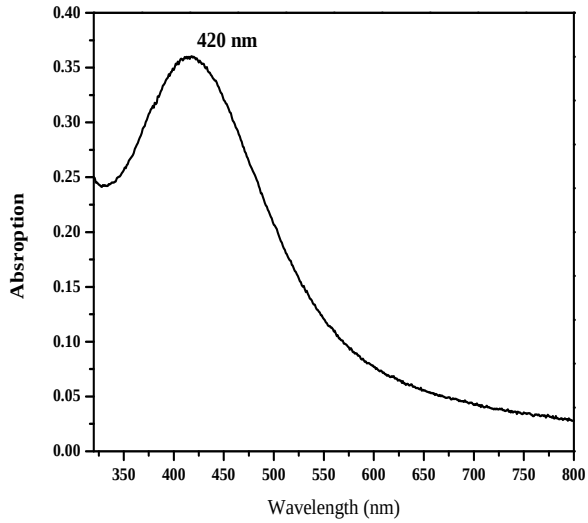

Fig.1. UV-Visible spectrum of biosynthesized AgNPs and its Plasmon excitation upon the interaction with *B. kewensis* plant leaf extract.

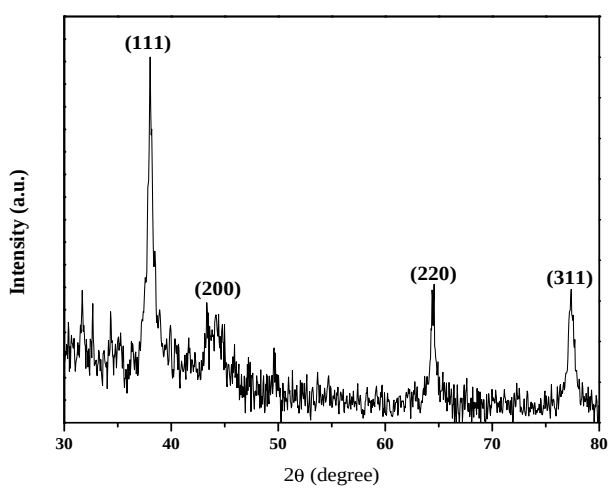

Fig.2. XRD spectrum of green synthesized AgNPs from *B.kewensis* plant leaf extracts.

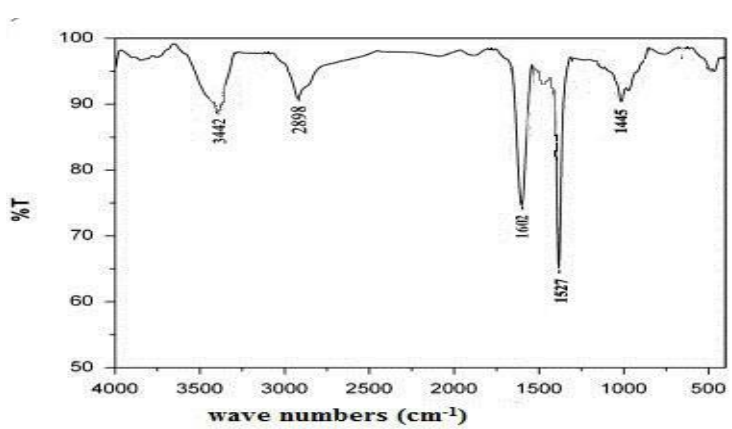

Fig.3. FTIR spectrum of green synthesized AgNPs from *B.kewensis* plant leaf extracts.

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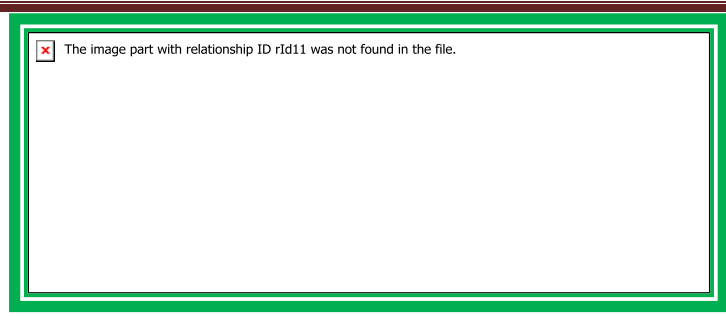

Figure 4 (a-b) FESEM image of high and low magnification of green synthesized AgNPs from *B.kewensis* plant leaf extracts.

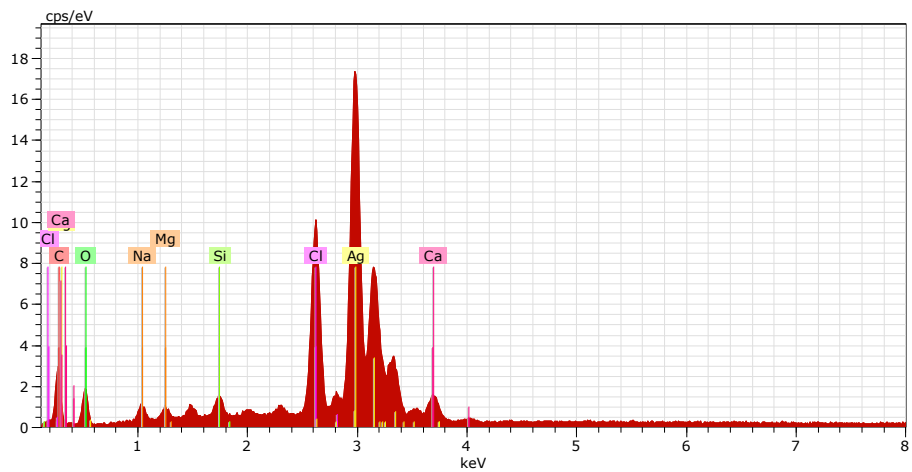

Fig.5.EDX spectrum of green synthesized AgNPs from *B.kewensis* plant leaf extracts.

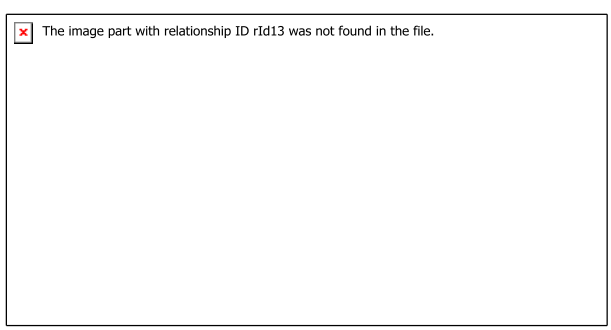

Fig.6. Antibacterial activity of three pathogenic bacteria against plant leaf aqueous extract and AgNPs. PE- Plant Extract; c- Control (Tetracycline); AgNPs- Disc prepared with silver nanoparticles.

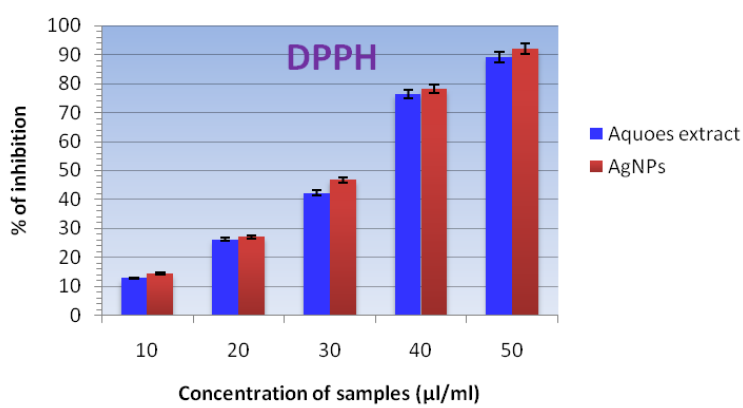

Fig. 7. Estimation of DPPH in phytosynthesized nanoparticles and plant leaf extact.