



## Synthesis of Silver Nanoparticles by *Ocimum basilicum* Seed Extract and its Application in H<sub>2</sub>O<sub>2</sub> Sensing

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### Abstract

Plant extract mediated nanomaterials synthesis is one of the emanating fields of nanotechnology with voluminous applications in the physics, chemistry, biology, medicine, etc. Although different chemical techniques are on the brink of development, the rising concern for environmental contamination opens the path for biological synthesis as most of the chemical routes need toxic chemicals. The current piece of the study intended to synthesize silver nanoparticles by aqueous extract from the seed of the medicinal plant, *Ocimum basilicum*, under ambient conditions. A fixed ratio of plant extract to metal ion was prepared, and the color change was observed which proved the formation of nanoparticles. TEM analysis revealed silver nano-particles existed in a quasi-spherical shape with an average size of 11 nm in diameter. Moreover, the H<sub>2</sub>O<sub>2</sub> sensing capacity of biologically synthesized AgNPs has also been studied.

**Keywords:** *Ocimum basilicum*; Silver Nanoparticles; Characterization; H<sub>2</sub>O<sub>2</sub> Sensing

### Introduction

The emergence of green nanotechnology is gaining interest these days due to their ecologically sound and cost-effective methods of synthesizing nano-materials. Metallic nanoparticles are attracting researchers attention due to their unique optical, electrical, and biological characteristics, making them suitable for various applications such as bio-sensing, imaging, optical spectroscopy, drug delivery, and catalysis [1]. Among the array of nanoparticles, silver nanoparticles (AgNPs) are believed to be full of promise in the nanotechnology industry and find a role in catalysis, material, energy and biomedicine [2-7]. These properties generally rely on the dimensions, shape, structure, and capping layer of nanoparticles [8, 9]. The preferred route for the synthesis of AgNPs in the chemical reduction of ionic silver in the presence of reducing and stabilizing agents [10, 11]. The environmental hazards associated with these chemicals paved the way for the green route of the synthesis of silver nanoparticles [12].

Moreover, the green route of synthesis is economical, devoid of deploying unsafe substances, high temperature, pressure and energy consuming processes that have detrimental effects in the medical field [13, 14]. The deployment of plant extracts in nanoparticle synthesis is advantageous over microbes due to the

ease of scale-up production, a small level of bio-hazard, and lack of need for complicated procedures in cell culture maintenance [15]. The deployment of plants and their parts in the synthesizing nanomaterial has been explored as a route of green synthesis of nanoparticles because extract derived from them possess different biomolecules including proteinaceous enzymes, phenolics, flavonoids, saponins, terpenoids, alkaloids which are responsible for reducing bulk or ionic form into nano-dimension of corresponding material [16]. Biomolecules such as flavonoids, proteins, and polysaccharides are responsible for the reduction of Ag<sup>+</sup> to Ag<sup>0</sup> [17]. In the recent past, the extract of different plant species such as *Eucalyptus chapmaniana*, *Arbutus unedo*, *Sesbania grandiflora*, *Allium sativum* has been deployed in the synthesis of Ag Nps [18-21]. *Ocimum basilicum* seeds are sources of a wide range of secondary metabolites such as phenolics and polyphenols, including flavonoids and anthocyanins [22]. These seeds are the plentiful insoluble fiber that maintains gut health, cholesterol level, blood sugar, and appetite. *Ocimum basilicum* seeds are rich in flavonoids which have antioxidant, anti-bacterial, anti-inflammatory and anti-cancerous properties. The Presence of these biomolecules makes these seeds an excellent candidate in the synthesis of nanoparticles. The remarkable characteristics of AgNPs such as small size,

larger surface-area-to-volume ratio, and spherical shape, facilitate attachment with the microbial cell wall, thereby imparting more significant antimicrobial effects [23].

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) a strong oxidant that finds potent applications in food, cosmetics, wood pulp, and pharmaceutical industries. Despite relevance, its presence in trace amounts may be accounted for the numerous health and environmental hazards due to toxicity. Thus, there is a need for designing a competent colorimetric sensor that can rapidly detect H<sub>2</sub>O<sub>2</sub> in various samples [24]. Moreover, Selvaraj and co-workers also reported the sensing capacity of AgNps synthesized by deploying *E.coli* [25]. The capability of AgNPs in reducing hydrogen peroxide resulting in their decolorization has been investigated for the fabrication of simple and cost-effective colorimetric H<sub>2</sub>O<sub>2</sub> sensor which can swiftly sense hydrogen peroxide.

### Materials and Methods

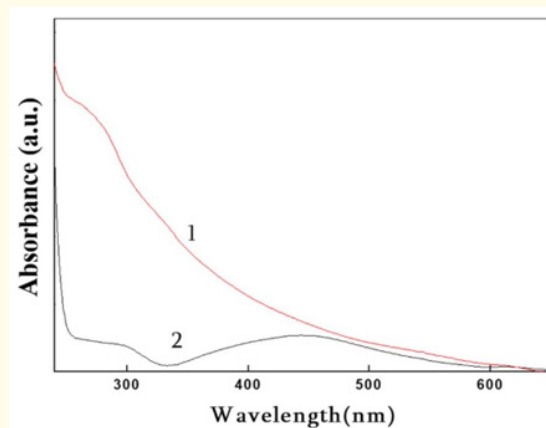
Silver nitrate (AgNO<sub>3</sub>) was purchased from Rankem fine chemical laboratory Ltd (RFCL). Ethanol was purchased from Merck (Germany). All glassware is of Borosil company. *Ocimum basilicum* seed was brought from Ajmal Khan Tibbiya Collage AMU, Aligarh. All reagents, used in the synthesis work, were of analytical grade and were purchased from commercial sources and utilized without further purification. Firstly, we take 80ml distilled water and 20ml ethanol in a conical flask. A certain weight of *Ocimum basilicum* seeds (5gm) was boiled in 100ml solution (water + ethanol) for 30 minutes to obtain the seeds extract through filter paper. The filtered extract was used as a reducing agent. This boiled extract was refrigerated and used for the experimental procedure.

1mM solution of silver nitrate (AgNO<sub>3</sub>) from stock solution was prepared using sterile deionized double distilled water. 1ml of *Ocimum basilicum* seed extract was added to 5ml of (1 × 10<sup>-3</sup>M) aqueous silver nitrate (AgNO<sub>3</sub>) solution, and the reaction was left to take place at ambient conditions. The observed change in color from transparent to light yellow brown color indicating that the formation of Ag Nps. The as-prepared solution was then used to inspect the formation of nanoparticles by UV-Vis spectroscopy, Transmission electron microscopy (TEM), X-ray diffraction (XRD) analysis and Fourier transform infrared (FTIR) spectroscopy. The optical property of AgNPs was determined by the Cary series UV-VIS-NIR spectrophotometer system (Aligent Technologies) was operated at a resolution of 1 nm in the wavelength range of 200-800 nm. To find out the morphology of Ag Nps, measurements were performed on the JEOL JEM 2100F transmission electron microscope

(TEM) operated at an accelerating voltage of 200 KV at university Sophisticated Instrument Facility, AMU. The samples T.E.M were prepared by drop coating on to a carbon copper grid. To confirm the crystallinity of synthesized Ag Nps, XRD data were recorded using RigakuMiniflex-II X-ray diffractometer equipped with high-intensity Cu-K $\alpha$  radiations ( $\lambda = 1.5406 \text{ \AA}$ ) operated at a voltage of 30 kV and a current of 15 mA at the scan rate of 2°/min in the 2 $\theta$  range of 30-80°. The chemical constituents in the bio-synthesized silver nanoparticles was studied by using FTIR spectroscopy measurement on the sample using a KBr pellet was performed by using a Perkin-Elmer Spectrum One instrument. The spectrometer was operated in the diffuse reflectance mode at a resolution of 2 cm<sup>-1</sup>. To obtain good signal to noise ratio, 128 scans of the powder were taken in the range of 450-4000 cm<sup>-1</sup>. The colorimetric detection of H<sub>2</sub>O<sub>2</sub> via Ag Nps was done by adding 1ml H<sub>2</sub>O<sub>2</sub> in 3 ml Ag Nps in a test tube. The resulting solutions were monitored by UV-Visible spectroscopy analysis at different time intervals.

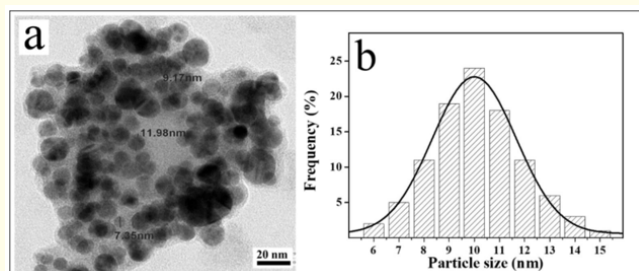
### Results and Discussion

Silver nanoparticles synthesized by using *Ocimum basilicum* seeds extract was characterized by UV-Vis absorption spectra. In figure 1, curve 1 shows a single peak maximum of seeds extracts at 243nm, which comes due to the presence aromatic compound present in the seed extract. In curve 2, which displayed peaks at 243nm (due to seed extract) and absorption at 421 which is characteristic absorption of Ag NPs [26] The emergence of a sharp absorption peak at 421nm indicates the formation of silver nanoparticles.



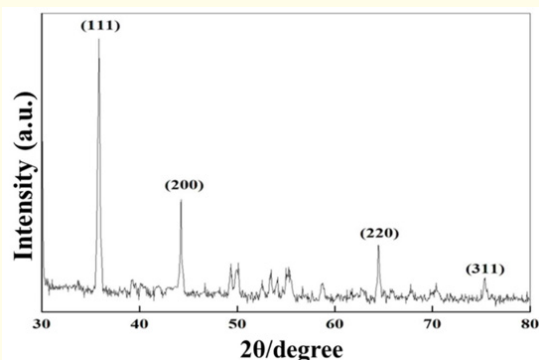
**Figure 1:** UV-Visible spectra (1) *Ocimum basilicum* seed extract (2) Ag Nps after reaction with plant seeds extract of the reaction mixture.

In figure 2a, a TEM micrograph of as-synthesized Ag nanoparticles is shown, which revealed that the nanoparticles are quasi-spherical in shape and capped with the seed extract. The obtained silver nanoparticles are free from aggregation. The particle size of Ag Nps is in range of 6-15 nm, in which most of the Nps are of 11 nm in size (Figure 2b). These Nps are relatively stable even after prolonged storage [27].



**Figure 2:** (a) TEM images of Ag nanoparticles obtained after reaction between seeds extract and AgNO<sub>3</sub> (b) Particle size histogram.

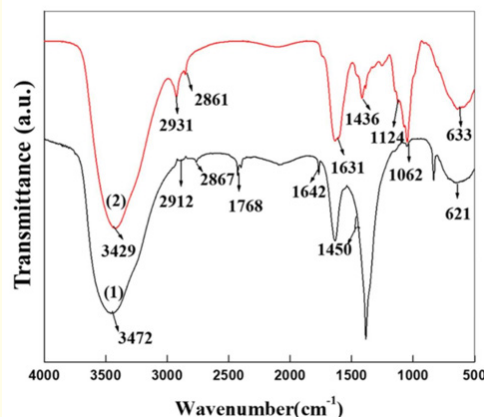
XRD analysis reveals the crystalline nature of Ag NPs. The diffracted intensities were recorded from 30° to 80° (2θ), XRD peaks were observed corresponding to reflection planes (111), (200), (220), (311) at 38.52°, 43.23°, 64.84° and 77.32° of metallic silver nanoparticles [28]. All the peaks are well-matched with the standard JCPDS file No 040783.



**Figure 3:** Powder XRD pattern of drop cast film of Ag nanoparticles on glass substrate after reaction with the herbal extract of *Ocimum basilicum* seeds extract.

FTIR measurements were accomplished to identify the presence of various functional groups in biomolecules responsible for

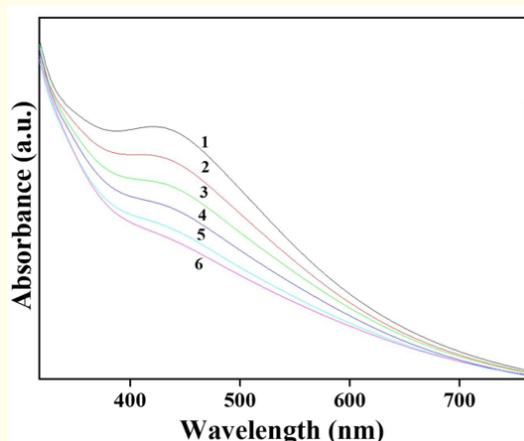
the bioreduction of Ag<sup>+</sup> and capping/stabilization of silver nanoparticles. FTIR spectrum of synthesized Ag NPs was shown in figure 4. The band at 3472cm<sup>-1</sup> in the spectrum corresponds to O-H stretching vibration indicating the presence of alcohol and phenol. The band at 2912cm<sup>-1</sup> and 2867cm<sup>-1</sup> region arising from C-H stretching of the aromatic compound were observed. The band at 1768cm<sup>-1</sup> was assigned for (C-C) stretching (non-conjugated). The band at 1642 cm<sup>-1</sup> in the spectra corresponds to C-N and C-C stretching, indicating the presence of protein [29]. The band at 1450cm<sup>-1</sup> was assigned for N-H stretch vibration present in the amide linkage of the proteins. These functional groups have a role in the stability/capping of Ag NPs [30]. The band at 1450 and 1062cm<sup>-1</sup> were assigned for N-H and C-N (amines) stretch vibrations of the proteins, respectively. FTIR spectrum of *Ocimum basilicum* seed aqueous extract was shown in figure 4. The band at 3429cm<sup>-1</sup>, 2931cm<sup>-1</sup>, 2861cm<sup>-1</sup>, 1631cm<sup>-1</sup>, 1436cm<sup>-1</sup>, 1342cm<sup>-1</sup>, 1124- 911 cm<sup>-1</sup> corresponds to polyphenols, carboxylic acids its derivative (C=O), N-H stretching and C=N stretching of aliphatic amines of seed extracts.



**Figure 4:** FTIR spectra recorded from as-synthesized Ag nanoparticles (curve 1) and seeds extract (curve 2).

The hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) sensing was studied on the reaction of Ag NPs with H<sub>2</sub>O<sub>2</sub>, which lead to the change of the color of the solution containing Ag Nps from yellowish to colorless. The presence of Ag NPs in the solution results in an influential absorption band at 421nm. The UV-Vis spectra of samples containing H<sub>2</sub>O<sub>2</sub> concentrations are shown in figure 5. Ag NPs can detect the presence of hydrogen peroxide H<sub>2</sub>O<sub>2</sub> in a sample was affirmed by adding 1 ml of 20 mM H<sub>2</sub>O<sub>2</sub> to 3 ml Ag NPs solution. The UV-Vis spectrum was recorded as a function of time at regular intervals. The decreasing trend in the absorbance peak of Ag NPs with the time increased, and eventually the characteristic peak of silver

nanoparticles at 421nm disappeared [31]. This corroborated silver nanoparticles' ability to decompose hydrogen peroxide and thus provide a means of detecting its composition. Therefore, Ag NPs in hybrid will be etched from Ag<sup>0</sup> to Ag<sup>+</sup>. So, the concentration of Ag<sup>0</sup> will decrease, leading to the fading of the Ag NPs solution and then to colorless after adding H<sub>2</sub>O<sub>2</sub>. The above behavior provides a potential for quantitative detection of H<sub>2</sub>O<sub>2</sub> by measuring the Ag NPs surface plasmon resonance reduction at 421 nm. The correlation between the concentration of hydrogen peroxide and a decrease in the absorbance of silver nanoparticles as a function of time could be used as a measure to detect the H<sub>2</sub>O<sub>2</sub> in unknown samples rapid.



**Figure 4:** UV-Visible absorption spectrum of biosynthesized Ag Nps after adding H<sub>2</sub>O<sub>2</sub> at different intervals of time; 0 minute (curve 1), 1 minute (curve 2), 2 minutes (curve 3), 3 minutes (curve 4), 5 minutes (curve 5), 10 minutes (curve 6).

## Conclusion

In conclusion, we developed a simple green protocol for the synthesis of noble metal nanoparticles (Silver) from *Ocimum basilicum* plant seed aqueous extract. Synthesis of Ag Nps was studied through different characterization techniques. The primary confirmation of color changes from light colorless to yellowish-brown color was observed at 421nm and recorded by UV-Vis spectroscopy. FTIR results proved that bioactive compounds from *Ocimum basilicum* plant seed aqueous extract are responsible for silver bioreduction. The Ag Nps formed were in the average size range of 11nm with spherical morphology. X-ray diffraction pattern corroborated the crystalline nature of Ag Nps. The sensing capacity of Ag Nps towards H<sub>2</sub>O<sub>2</sub> was also demonstrated. Hence the Ag Nps

formed by this green synthesis method can be used as a probe for the detection of hydrogen peroxide in different samples.

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