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# Optimization of Reaction Conditions for Green Synthesis of Silver Nanoparticles Using *Coffea arabica* Leave Extract

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Present study describes a rapid and eco-friendly synthesis of silver nanoparticles (AgNPs) using *Coffea arabica* leaf extract in single process via green Chemistry. We have successfully synthesized *AgNPs using Coffea arabica* leaf as a reductant and a capping or stabilizing agent through green chemistry. In the typical synthesis of AgNPs, *Coffea arabica* leaf extract was added into aqueous solution of $AgNO_3$ in a conical flask at $80^0C$ temperature. Different reaction concentrations of *Coffea arabica* extract and $AgNO_3$ solution were subjected. AgNPs were synthesized by using aqueous silver nitrate solution as a precursor with aqueous solution of *Coffea arabica* leaf extract as the reductant and stabilizing agents. The AgNPs were characterized using Fourier transform infrared spectroscopy (FTIR), X-Ray Diffraction (XRD), Atomic Absorption Spectroscopy (AAS) and UV-Vis spectroscopy. The optimum conditions for this process were a

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temperature of *80°C*, pH 9, and reactant ratio 1:4. XRD results reveal that, the *green synthesis* yields stable, face centered cubic structure AgNPs with a sizes range of 39.664-24.708 nm. Preparation of AgNPs using plant extracts are eco-friendly, biocompatible and cost effective. The synthesis method applied in this study involved the application of the green chemistry principles which emphasizes on the use of nontoxic solvents and reagents to protect the environment. AgNPs have a vital role in different nanotechnology-based processes because of their unique characteristics and can be exploited on a large scale for medical application.

*Keywords: Coffea arabica; green synthesis; aqueous extract; nanoparticles.*

## 1. INTRODUCTION

"Green chemistry is a significant field of research as it offers simple, cost-effective, easy, biocompatible and eco-friendly methods for the synthesis of nanomaterials *in the* field *of nanotechnology"* [1]. "Nanotechnology" is the newest and one of the most promising and active areas of modern research. The technology deals with the design, synthesis, and manipulation of particles size ranging from 1–100 nm [1]. "Within this size range, the chemical, physical, and biological properties change in the fundamental way of both individual atoms and their corresponding bulk material" [2]. This very small size increases the surface area-to volume ratios of particles. The nanoparticles synthesized using plant extract have gained huge consideration in recent years due to their remarkable properties and wide range of applications in catalysis [3], plasmonic [4], optoelectronics [5], biological sensor [6], water treatment, pharmaceutical applications [7], and agriculture and crop protection [8]. "Stunning growth in this emerging technology has opened novel fundamental and applied frontiers, including the synthesis of nanomaterial and utilization of their physicochemical and optoelectronic properties" [9]. "The application of nanotechnology has increased in large number of areas such as optics, mechanics, chemical industries, space industries, electronics, energy science, single electron transistors, light emitters, nonlinear optical devices, photo-electrochemical, catalysis, biomedical, cosmetics, drug delivery, and food and feed" [10-14].

"Due to their exceptional biological and physicochemical properties, metal nanoparticles (NPs) have devoted particular attention to their use in emerging constituents for various applications" [15]. "Among several metal nanoparticles, silver nanoparticles (AgNPs) are one of the most dynamic and interesting nanomaterials that have gained significant attention in the arena of nanotechnology" [16]. "AgNPs and their nanocomposites have created marvelous potential and noteworthy applications in diverse fields of nanotechnology, particularly in biomedical therapeutic researches, MRI contrast agents, drug delivery, and biomedical devices for the detection of numerous alarming diseases or complications" [17]. "Moreover; AgNPs have a wide range of use because of their unique characteristics such as optical, electrical and magnetic properties, which can be incorporated into antibacterial, antiviral, and antifungal applications, composite fibers, biosensor materials, cosmetic products, food industry uses, and electronic components" [18,19]. "AgNPs are also reported as medical and pharmaceutical agents that have directly encountered by a human system in such products as shampoos, detergents, soaps, toothpaste, and cosmetics" [20]. The biomedical use of AgNPs includes their application as antibacterial [21], antifungal [22], anti-inflammatory [23,24], antiviral [25], and anti-diabetic agents [26].

"In the intensive search for a good biological agent for synthesizing metal nanoparticles, several biological entities from microbes to plants and animal products have been given much attention" [27-29]. "Among the different bio-tools used to produce nanoparticles, plant extracts are given high priority" [30-32]. "Green synthesis routes using various plants have produced active materials that have greater potential against gram-positive and gram-negative microorganisms and different cancer cell lines and exhibit higher antioxidant effects than those produced by chemical and thermal physical methods" [33-35].

In recent studies, green synthesis of AgNPs using extracts of Garcinia mangostana [36], Lepidium draba [37], Crocus Haussknechtii Bois [38], Mimusops elengi [39], Boerhaaviadiffusa [40], Meliadubia leaf [41], Pistacia atlantica [42], Momordica cymbalaria [43], Mentha aquatica [44], Parquetina nigrescens and Synedrella nodiflora [45], Terminalia chebula [46], Costus afer [47], Ormocarpum cochinchinense [48], Callistemon citrinus [49], and Tectona grandis

[50] have been reported. *In addition to the previous reports, a wider range of applications of bio-inspired synthesized AgNPs has been reported.*

*The current study focuses on the* synthesis and characterization of AgNPs from *Coffea arabica* leaf extract*. Green synthesis was a method utilized for the preparation of AgNPs and was characterized* via*: UV-visible spectroscopy, Atomic Absorption Spectroscopy (AAS), X-ray di*ff*raction (XRD), Fourier transform infrared spectroscopy (FTIR), and Scanning Electron Microscope (SEM) analysis.* The results of this study will have a significant role in the scientific knowledge of plant mediated synthesis of nanoparticles. The synthesis method applied was green chemistry *which* emphasizes on the use of nontoxic solvents and reagents to protect the environment. The novelty of this study is that it is the first report of the synthesis of AgNPs using *Coffea arabica* . As far as our knowledge is concerned, there is no any report regarding the green synthesis of AgNPs using *Coffea arabica* leaf extract.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of the Leaf Extracts

For the preparation of the aqueous extract, fresh green leaves of *Coffea arabica* was used. Some amount of *Coffea arabica* leaves were weighed, washed with tape water followed by distilled water to remove all the dirt. Then the clean leaf was shade dried, grounded using mortar and pestle, and boiled in 250 ml of distilled water at $80^0$C for 3 minutes. Finally, the solution was cooled and filtered with What-man No.1 filter paper and thus, the aqueous extract was kept at $4^0$C for AgNPs preparation [51].

### 2.2 Preparation of $AgNO_3$ Solutions

$AgNO_3$ was carefully weighed using analytical balance and then transferred into 1000 ml volumetric flask that contained 400 ml of distilled water. The solid material was completely dissolved and distilled water was added to fill up to the mark. A 0.004 M solution of $AgNO_3$ was prepared in an amber bottle and stored in a cool and dry place [52].

### 2.3 Green Synthesis of AgNPs

In the typical synthesis of AgNPs, aqueous *Coffea arabica* leaf extract (20 g/250 ml) was added into the aqueous solution of 4 mM $AgNO_3$ in a conical flask at 80 $^0$C. Different reaction concentrations of *Coffea arabica* extract and $AgNO_3$ solutions were subjected. Ag ion was reduced into Ag metal. The prepared AgNPs were characterized using XRD, FTIR, SEM, AAS and UV-Vis spectroscopy [53].

### 2.4 Spectrophotometric Analysis of the Samples

UV-Vis spectroscopy refers to the absorption/emission in the range of UV-Vis spectral region. Usually, 350–700 nm light is used for the characterization of 2–100 nm sized metal nanoparticles [54]. UV–vis spectroscopy is an instrument that can be used to evaluate the formation and stability of AgNPs in aqueous solution [55]. Spectrophotometric absorption measurements in the wavelength ranging from 380 up to 500 nm commonly use to characterize silver nanoparticles [56]. In this work a double beam UV-Vis instrument with a scan rate of 4800 nm/min at a wavelength range of 350-700 nm was used and the spectroscopic analysis was done using Cary 60 version 2.

### 2.5 FTIR and XRD Analysis of AgNPs

FTIR studies were carried out using a shimadzu spectrophotometer (model 100, Japan). The samples were synthesized using KBr pellet method and *Coffea arabica* leaf extract as well as the synthesized Ag nanoparticles were analyzed for the presence of bio-functional moieties under optimized conditions. FTIR spectra of AgNPs were recorded at a resolution of 2 $cm^{-1}$ in transmission mode between 3500 and 650 $cm^{-1}$. XRD pattern of synthesized nanoparticles was also done by using DX-2700 X-Ray Diffractometer. The X-ray source was obtained in Cu-$K_\alpha$ radiation with 0.154 nm wavelength. The XRD system was run at 30 mA current and 40 KV voltage and the nanomaterials were scanned in the range of 3 - 80℃.

### 2.6 AAS Analysis of AgNPs

The conversion of Ag ion to Ag metal can be inferred with this measurement. The aliquant sample was withdrawn and shaken at regular interval during the reaction. The supernatant solution contains the unreacted $AgNO_3$ for the reason that Ag ions are so much smaller than Ag metal and the pellets contain AgNPs. The supernatant solution was examined using AAS to identify the concentration of Ag ions. The reduction rate of the concentration of Ag ion was exhibited the conversion of Ag ion into Ag metal.

## 3. RESULTS AND DISCUSSION

### 3.1 UV-Visible Spectroscopy Characterization

#### 3.1.1 Effect of temperature on the synthesized AgNPs

Temperature is a physical factor that plays a great role in controlling the nucleation reaction of nanoparticles fabrication. The absorbance of silver nanoparticle was recorded at a temperature 25℃, 50℃, 60℃, 70℃ and 80℃. On the basis of UV-Vis studies better synthesis was demonstrated at 80°C.

UV-visible absorption spectra have been used to prove its significance and sensitivity towards silver colloids formation since AgNPs display an intense absorption peak as a result of the Surface Plasmon. As shown in Fig. 1, a wide peak was observed at 450 nm and 25℃ for the reaction mixture. Broadening of band attributed to the agglomeration and increase in size of the particles [57]. Increase in absorbance increases the temperature from 25 to 80℃ and the bands become narrow. Moreover; nanoparticles synthesized at higher temperature increase the rate of formation of AgNPs, retarding the secondary reduction process. Others study by [58] also reported similar result and attributed the trend to the increase in solubility of the phenolic at higher temperature. Increasing the absorbance of the reaction mixture increases the incubation temperature markedly showing higher productivity of AgNPs, at elevated temperature. The sharpness of absorbance peak depends on the size of the nanoparticles. The nanoparticle size become smaller when the temperature is higher, resulting in sharpness of the Plasmon resonance band of silver nanoparticles [59].

#### 3.1.2 Effect of pH on the synthesized AgNPs

Preparation of AgNPs using *Coffea arabica* leaf was conducted over a pH range of 3–11. Fig.2 shows that the UV-Vis spectra of Ag nanoparticles extracted from *Coffea arabica* leaf and the effect of the variation in pH.

The UV-Vis absorption peak was very wide at pH five as indicated in Fig. 2. This wide result may be because of increase in AgNPs size. The presence of larger nanoparticles and platelets at lower pH can be attributed to the uncontrolled nucleation as well as aggregation because of the improved interaction of negatively charged ions. Another study was also reported, at low pH, the aggregation of AgNPs to form larger nanoparticles was believed to be favored over the nucleation to form new nanoparticles [60]. An efficient AgNPs were prepared at pH 9 and the size remained smaller but agglomeration AgNPs was noticed at pH 11, therefore pH 9 was the optimal value for efficient AgNPs synthesis. Our result agreed with what was reported by [61]. At higher pH, however, the large number of functional groups available for silver binding facilitated a higher number of Ag to bind and subsequently form a large number of nanoparticles with smaller diameters. In addition at higher pH the shape of the nanoparticles formed were more of spherical in nature rather than ellipsoidal. There was no formation of silver nanoparticles at pH < 5, this might be due to the instability of the nanoparticles at lower/acidic pH [62]. Acidic conditions avoid the formation of AgNPs whereas, basic conditions enhance the formation of AgNPs. This result confirmed the very important role played by pH in controlling the shape and size of the AgNPs synthesis.

#### 3.1.3 Effect of reaction time on the synthesized AgNPs

The reaction between reducing agent and Ag ion was run for 6, 12, 24 and 48h at room temperature. The color change of the solution from yellow to brownish-yellow and then to deep brown indicates that the synthesis of AgNPs when 4mM $AgNO_3$ was added to *Coffea arabica* leaf extract at different time interval. Fig. 3 shows the UV-Vis absorption spectra of the colloidal solution. It should be noted that the intensity of the color of the reaction mixture was directly proportional to the incubation time due to excitement in the surface Plasmon resonance phenomenon. The maximum absorbance was observed at 12h time interaction. When the absorbance was increased, the interaction time of Ag ions with *Coffea arabica* leaf extract was also increased. UV-Vis spectra in Fig. 3 shows that the best synthesis was obtained at 12h of incubation. The obtained results demonstrated that the higher the absorbance the more AgNPs formed as the reaction time increases to 12h and similar results were also reported by [63]. An optimum period is required due to the unsteadiness of the AgNPs synthesis, as agglomeration after optimum time interval resulting in larger sizes of particles. As absorption increases, the incubation time increases from 6 to 12h. Agglomeration of AgNPs was occurred after the optimum time due to the instability of the nanoparticles, therefore, maximum time was required to form larger particle size [64].

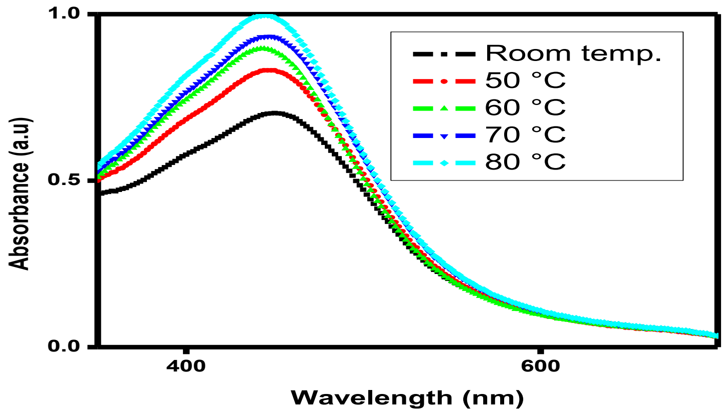


**Fig. 1. UV-Vis absorption spectra of the synthesized AgNPs at different temperatures**

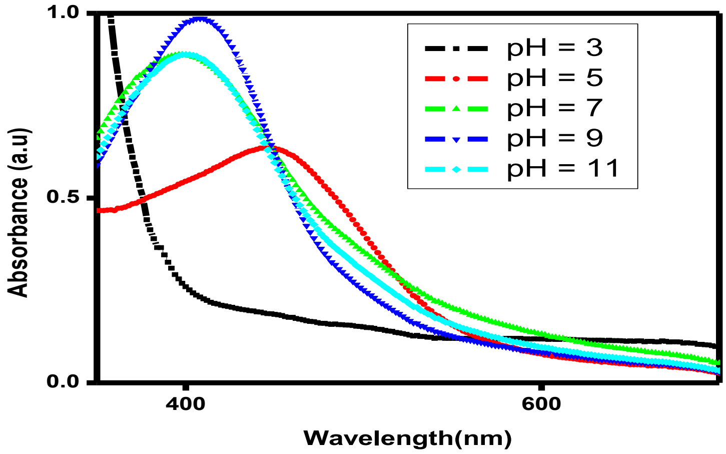


**Fig. 2. UV-Vis absorption spectra of the synthesized AgNPs at different pH values**

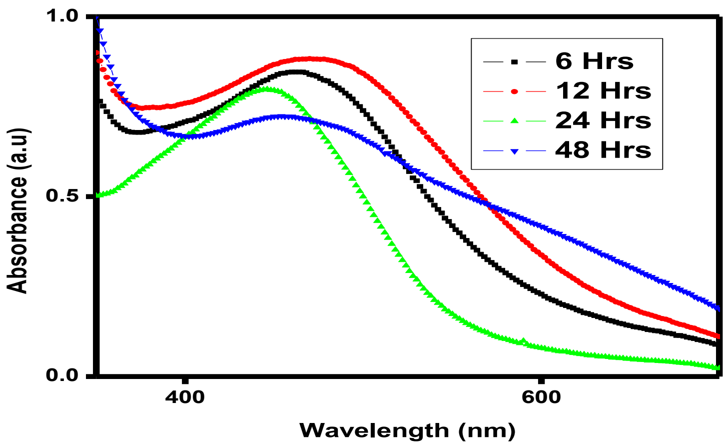


**Fig. 3. UV-Vis absorption spectra of the synthesized AgNPs at different reaction time**

### 3.1.4 Effect of concentration on the synthesized AgNPs

The absorbance peak (454 nm) of *Coffea arabica* leaf extract was broad and less intense as shown in Fig. 4 at 5 g/250 ml leaf extract concentration. However, as the *Coffea arabica* leaf extract concentration increases gradually from 5 g/250 ml to 20 g/250 ml, the absorbance peak becomes narrower and more intense. There was gradual increase in absorbance as the *Coffea arabica* leaf extract concentration increases. Based on the UV-Vis studies, the synthesis of AgNPs was better demonstrated at 20 g/250 ml. At lower extract concentrations, a lesser number of nucleation sites were present so more reduction were taken place at single nuclei leading. At higher concentration the polyphenols in the *Coffea arabica* leaf extract were effectively reduced from Ag-ions to $Ag^0$ and provide enough capping agent for the stabilization of the synthesized nanoparticles through steric hindrance. Thus preventing their aggregation, which probably leads to the formation of smaller particles at a higher concentration [65]. These results were agreed with what was reported before [66].

### 3.1.5 Effect of silver ion concentration on the synthesized AgNPs

Various concentration such as 0.5, 1, 2, 3 and 4mM $AgNO_3$ were taken for the optimum preparation of nanoparticles. Interestingly 4mM concentration supported rapid formation compared to the other concentrations (Fig. 5). When the concentration of $AgNO_3$ was increased the number of AgNPs were risen. When $AgNO_3$ concentration was increased from 0.5 up to 4mM, the yield of AgNPs were also increased. Hence 4mM concentration of $AgNO_3$ was chosen for further experimentation. The concentration of metal ion has an essential role in the nanoparticles production. Similar effect of varying concentration of silver salt on yield, size and disperse of AgNPs was reported by others [67].

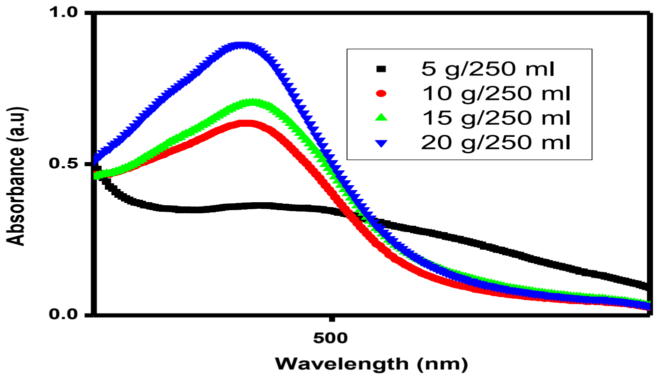


**Fig. 4. UV-Vis absorption spectra of the synthesized AgNPs at different concentration**

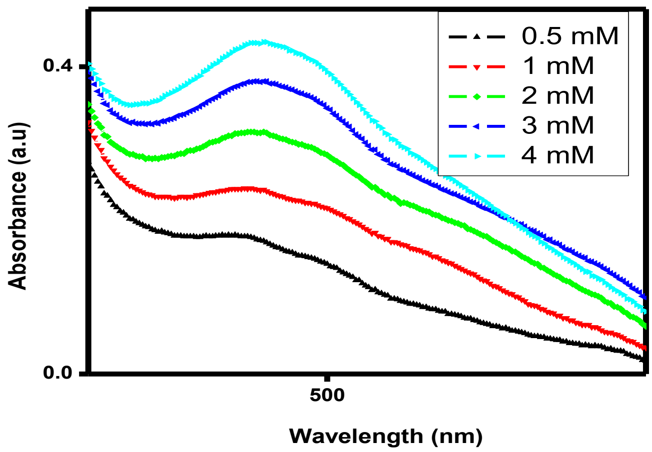


**Fig. 5. UV-Vis absorption spectra of the synthesized AgNPs at different Ag ion concentration**

### 3.1.6 Effect of reactants ratio on the synthesized AgNPs

Different volume (10, 20, 30, 40 and 50 ml) of plant extracts were added to 4mM $AgNO_3$ aqueous solution at room temperature. The solutions were characterized using UV-Vis after 12h. Fig. 6 shows that the UV-Vis spectra of AgNPs at different volume of *Coffea arabica* leaf extract (10-50ml). A maximum absorbance was observed at 20ml *Coffea arabica* leaf extract with narrow peak. The absorbance increases with increasing the volume *Coffea arabica* leaf extract, and reached maximum at 20ml, then start to decrease from 30 – 50 ml. UV-Vis spectra (Fig. 4) indicated that 20ml *Coffea arabica* leaf extract yielded best result. The optimal concentration of the reactant ratio was 1:4. The obtained results support similar finding reported by [68]. The stability of the colloidal solution was influenced by the absorbing species present and concentration of reducing agents [69].

### 3.1.7 Effect of light on the synthesized AgNPs

Effect of light on the rate of AgNPs synthesis clearly demonstrated better synthesis in sunlight compared with other light sources such as moonlight and dark room. Sunlight was an essential source for the synthesis of the nanoparticles and used as photosensitization of the molecules present in the plant extract. Fig. 7 shows that the UV-Vis absorption spectra of synthesized AgNPs at different light sources. When the reaction mixture was kept in dark overnight no color change was observed and the synthesis was delayed, similar findings were reported by others [70]. Current study showed that light is significantly executing for green synthesis of AgNPs from the leaf extract.

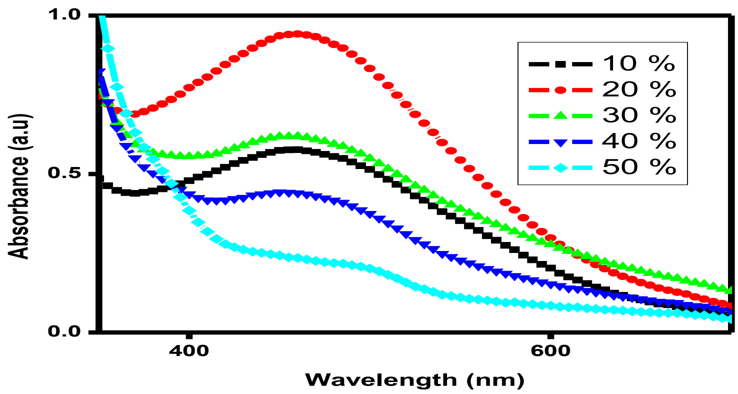


**Fig. 6. UV-Vis absorption spectra of the synthesized AgNPs at different ratio of reactants (v/v %)**

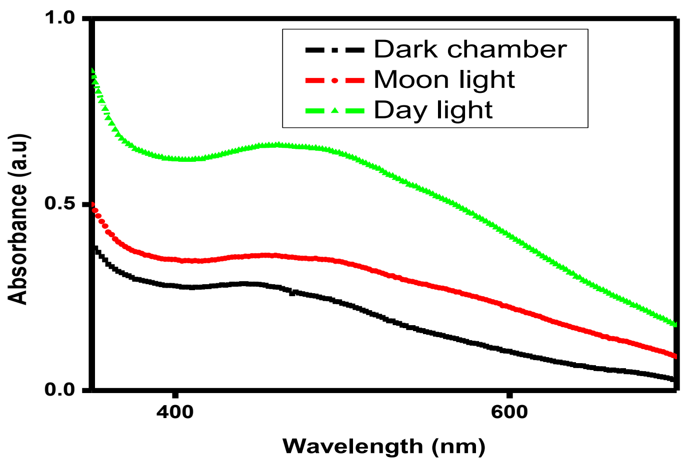


**Fig. 7. UV-Vis absorption spectra of the synthesized AgNPs at different light sources**

### 3.2 FTIR Analysis

Fig. 8 shows the FTIR spectra of *Coffea arabica* leaf extract and synthesized AgNPs. FTIR measurement of *Coffea arabica* leaf extract and synthesized AgNPs were carried out to identify the functional groups present and proteins surrounding as stabilizing agent. The sample contains alcoholic, phenolic and carboxylic groups indicating that broadband around 3440 $cm^{-1}$ corresponding to O–H stretching of hydroxyl group. Moreover; primary and secondary amines and amides were signified by N–H stretching. This band can be attributed to the non-dissociative adsorbed water molecules as well as the presence of N-H stretching can prove the presence of band at 1600 $cm^{-1}$ due to the vibration of water molecules. The peak around 1154 $cm^{-1}$ is ascribed to C–N stretching of aromatic and the bands at 900–700 $cm^{-1}$ correspond to primary and secondary amines and amides ($–NH_2$ wagging). The presence of ketones, aldehydes, quinines and esters can be shown by the peaks around 1600 $cm^{-1}$ assigned to the C=O vibration of carbonyl groups. The band at 2949 $cm^{-1}$ is attributed to C–H stretching of alkanes. The band at 2310, 2154, 1377 and 1018 $cm^{-1}$ attributed to the strong stretching of C–N aromatic and aliphatic amines. The peak at 1600 $cm^{-1}$ is attributed to C=C vibration of aromatic structure whereas the peak at 1274 $cm^{-1}$ is assigned to C–O stretching of phenolic groups. Another broadband centered around 1030 $cm^{-1}$ is attributed to the aromatic ethers and polysaccharides (C–O–C) stretch [71]. Carbonyl groups proposed the presence of OH functional groups and proteins show the presence of phenolic compounds in the *Coffea arabica* leaf extracts. After AgNPs formation, there were some shift of valuable peaks such as the O–H vibration from 3449.96 $cm^{-1}$ to 3448.75 $cm^{-1}$, N–H vibration from 2958.85 $cm^{-1}$ to 2949.21 $cm^{-1}$, 2725.46 $cm^{-1}$ to 2726..43 $cm^{-1}$, C–N vibration from 1456.28 $cm^{-1}$ to 1458.21 $cm^{-1}$ and 1154.42 $cm^{-1}$ to 1153.45 $cm^{-1}$, and disappearance of peaks 2154.52 $cm^{-1}$ indicating that reduction occurred.

### 3.3 XRD Analysis

The crystalline nature of the AgNPs formed was confirmed. XRD spectra of silver the synthesized nanoparticles are displayed in Fig. 9. The XRD pattern shows that, the structure of AgNPs is face-centered cubic [72]. The XRD analysis of synthesized silver nanoparticles had four peaks at 38.115°, 44.229°, 64.443°, 77.4° which corresponds to (1 1 1), (2 0 0), (2 2 0), (3 1 1) diffraction peaks. Sharp peak of (1 1 1) with high intensity was observed depicting thin film formation on the substrate. This agrees with previous report [73]. The sharp bands of Bragg's peak showed that the particles were in Nano form and stabilized by the reducing agents in the *Coffea arabica* leaf extract. Additionally, the Bragg's peak shows the representation of silver nanocrystals. The XRD pattern also shows additional peaks due to the organic compounds present in the *Coffea arabica* leaf extract and responsible for silver reduction and stabilization of the resulting nanoparticles [74]. Hence from the XRD pattern, it is clear that AgNPs formed by the reduction of Ag-ions using *Coffea arabica* leaf extract were essentially crystalline. The crystallite size was calculated by using the Debye Scherer's formula [75].

$$d = \frac{K\lambda}{\beta\, Cos(\theta)} \tag{3.1}$$

Where D is the average crystallite domain size perpendicular to the reflecting planes, k is Debye Scherer's constant (K=0.9), λ is the X-ray wavelength (0.15406 nm), β is udefull width at half maximum (FWHM), and θ is the diffraction angle. The average crystallite size according to Scherer's equation calculated was found to be 29.3 nm.

### 3.4 Scanning Electron Microscopy (SEM) Analysis

The morphology of the synthesized AgNPs were analyzed using FESEM as shown in Fig. 10. The FESEM image revealed that AgNPs spherical shapes were well dispersed. Some of the nanoparticles were oval shape due to this it was very difficult to measure the particle size. The biosynthesized AgNPs were disperesed in the solution as reported by Saba et al, [76].

### 3.5 Atomic Absorption Spectroscopy (AAS)

Fig. 11 shows the graph of Ag-ion concentration in the reaction mixture conducted by AAS. AAS analysis was done at regular time intervals and the conversion of Ag-ions into silver metal was observed. Initially, a standard solution of 5.0 ppm of $AgNO_3$ was prepared and analyzed using AAS. Then, Ag-ion concentration in the solution was monitored at regular time intervals after adding *Coffea arabica* leaf extract. Result indicated that decreasing in concentration of Ag-ions (5.0, 4.05, 4.03, 4.01 and 2.93 ppm) at 0, 5, 10, 15 and 20 min. respectively exhibiting the conversion of Ag-ion to Ag metal.

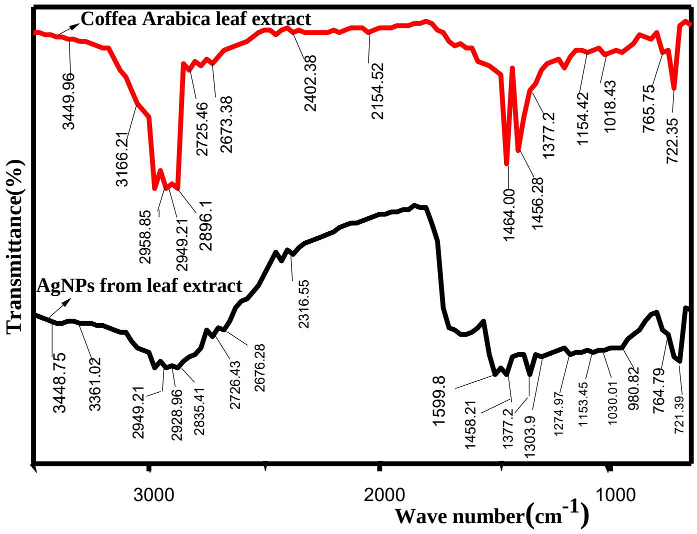


**Fig. 8. FTIR spectra of *Coffea arabica* leaf extract and synthesized AgNPs**

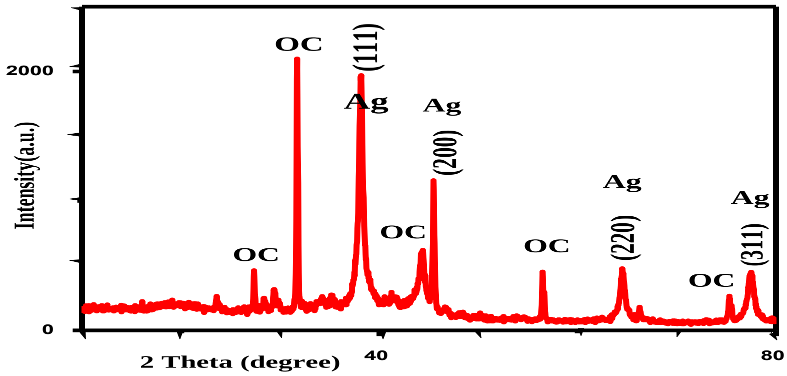


**Fig. 9. X-ray diffraction pattern of the synthesized AgNPs**

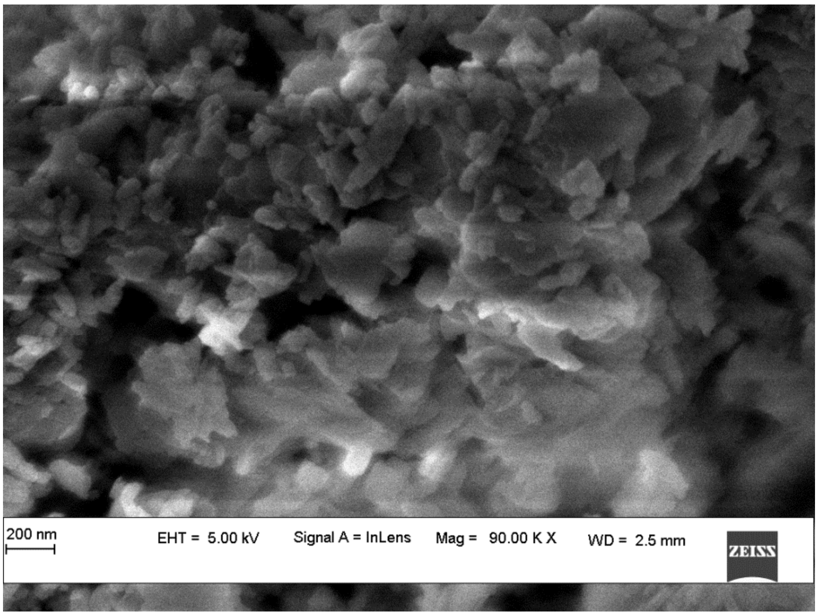


**Fig. 10. SEM diagram of the synthesized AgNPs**

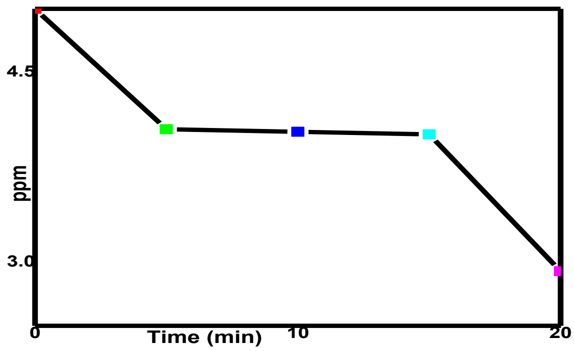


**Fig. 11. Graph of Ag-ion concentration in the reaction mixture**

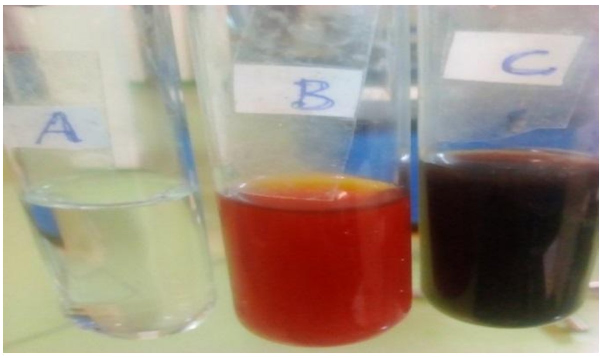


**Fig. 12. Color change of the leaf extract: A) 4mM $AgNO_3$ solution (colorless) B) aqueous extract of *Coffea arabica* leaves (yellowish brown) C) synthesized $AgNO_3$ (deep brown)**

## 4. CONCLUSIONS

*In conclusion,* present study describes a rapid and eco-friendly synthesis of AgNPs using *Coffea arabica* leaf extract in single process via green Chemistry. We have successfully synthesized *AgNPs using Coffea arabica* leaf as a reductant and a capping or stabilizing agent through green chemistry. The optimum conditions for this process were a temperature of *80 °C*, pH 9, and reactant ratio 1:4. XRD results reveal that, the *green synthesis* yields stable, face centered cubic structure AgNPs with a sizes range of 39.664-24.708 nm. Preparation of AgNPs using plant extracts are eco-friendly, biocompatible and cost effective. Generally AgNPs have a vital role in different nanotechnology-based processes because of their unique characteristics and *can be exploited on a large scale for medical application.* We believe that in the near future a greener and more biosynthesized AgNPs will open many new windows toward biomedical applications. Moreover; further research on AgNPs will provide opportunities in energy storage devices which will solve the current energy crisis globally.

## ACKNOWLEDGEMENTS

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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