X-RAY DIFFRACTION CHARACTERIZATION FOR OPTIMAL CRYSTALLITE AGGREGATES OF PHYTO-SYNTHESIZED SILVER NANOPARTICLES (AgNPs) BY *OCIMUM SANCTUM* L. LEAF EXTRACT

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Abstract

ABSTRACT

In this research work, the holy basil leaves were collected from Hpa-an Township, Kayin State and verified its botanical name at Department of Botany, Hpa-an University. Preparation of silver nanoparticles and characterization of prepared silver nanoparticles by X-ray diffraction are described in the present work. A cost effective and environmental friendly method has been carried out for phytosynthesis of silver nanoparticles (AgNPs) from different volumes (2, 4, 6, 8 and 10 mL) of silver nitrate solution (1.0 mM) as metal precursor by using different amounts (5, 10, 15, 20 and 25 μL) of *Ocimum sanctum* L. leaf extract as reducing agent without using other chemicals of reducing agents. Based on the volume of metal precursor solution, the phyto-synthesized silver nanoparticles were designated as AgNP-2, AgNP-4, AgNP-6, AgNP-8 and AgNP-10 for further characterization. From the X-ray diffraction characterization, it was observed that the XRD patterns of AgNP-8 and AgNP-10 from synthesized AgNPs only showed the optimal crystallite aggregates for cubic crystal system. Comparing with (JCPDS, DB card No. 00-006-0480) reference, the peaks at 2θ values of both AgNP-8 and AgNP-10 corresponded to the Miller indices ($h\ k\ l$) of cubic system of Ag nanoparticles. From indexing XRD patterns of cubic system, it could be confirmed that the optimal phyto-synthesized AgNP-8 and AgNP-10 were face-centered cubic (FCC) crystal system because of having Miller indices with all odd or all even. It was observed that the average crystallite sizes of AgNP-8 and AgNP-10 were found to be 57.65 nm and 49.82 nm by using Scherrer formula.

Keywords : Silver nanoparticles, phyto-synthesis, *Ocimum sanctum* L., XRD, aggregates, single crystallite size

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Introduction

Nanotechnology is a rapid growing science of producing and utilizing nano-sized particles. The uses of nano-sized particles are even more remarkable. They are mostly prepared from noble metals like silver, gold, platinum and palladium (Anuradha *et al.,* 2014). A number of approaches are available for the synthesis of silver nanoparticles, such as thermal decomposition, electrochemical, microwave assisted process and green chemistry. Many of the nanoparticle synthesis or production methods of nanoparticles involve the use of hazardous chemicals, low material conversions and high energy requirements (Logeswari *et al.,* 2015).

Biological methods have emerged as an alternative to the conventional methods for synthesis of nanoparticles. Synthesis of inorganic nanoparticles by biological systems make nanoparticles more biocompatible. Silver was recognized as a disinfecting agent; in its nanoparticles forms induce their ability in functions from medicine to culinary items. Human beings are frequently infected by micro-organisms such as bacteria, yeast, mold, virus, etc. Silver and silver ion based materials are usually used for their bactericidal and fungicidal activity.

The antimicrobial activity of silver is much superior to other metals, such as mercury, copper, lead, chromium and tin. Hence, silver and silver ion containing materials are used as prostheses, catheters, vascular grafts and as wound dressings in several biomedical applications.

Various methods, including physical and chemical methods were developed to synthesize metal nanoparticles, such as chemical reduction, electrochemical reduction, photochemical reduction etc. Biological method of nanoparticles synthesis using microorganisms, enzyme and plant or plant extract offers numerous benefits to offer a valuable contribution as eco-friendly technologies into nano-material science. In other hand, biological methods are free from the use of toxic solvents for synthesis of nanoparticles which are hazardous to the environment.

Nanoparticles are generally characterized by their size, shape, surface area and dispersity (jiang *et al*., 2009). The common techniques of characterizing nanoparticles are UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), powder X-ray diffraction (XRD) (Shahverdi *et al*., 2011) .

The UV-visible spectroscopy is a commonly used techniques (Pal *et al*., 2007). Light wavelengths in the 300-800 nm are generally used for characterizing various metal nanoparticles in size range of 2 to 100 nm. FTIR spectroscopy is useful for characterizing the surface chemistry (Chithrani *et al*., 2006). XRD is used for the phase identification of the crystal structure of the nanoparticles (Sun *et al*., 2000).

Among the various known biosynthesis methods, plant mediated nanoparticle synthesis is preferred as it is cost-effective, eco-friendly and safe for human therapeutic applications (Paramasivam *et al*., 2015). Nowadays, the use of different types of medicinal plants has been increased by traditional medical practitioners for the treatments of various types of diseases.

The plant extract may act both as reducing and stabilizing agent. The most common and important medicinal plant i.e., *Ocimum sanctum* L. which have different medicinal properties such as anticancer, antimicrobial, cardio-protective, antidiabetic, analgesic, antispasmodic, antiemetic, hepatoprotective, antifertility, adaptogenic and diaphoretic actions (Birendra and Panigrahi*,* 2015).

The health benefits of *O.sanctum* include skin care, dental care, relif from respiratory disorders, asthma, fever, lung disorders, heart diseases and stress. *O.sanctum* protects all sorts of infections from viruses, bacteria, fungi and protozoa.

The use of plant extract reduce the cost as well as we do not require any special culture preparation and isolation techniques. The advantages of using plants and their extracts for the synthesis of metal nanoparticle is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction (Gavade *et al*., 2015).

Materials and Methods

Collection of Holy Basil Leaves

Freshly green leaves of Holy basil (Kala-pin-sein) were collected from Hpa-an in Kayin State and identified by authorized botanists at Botany Department, Hpa-an University.

Preparation of Leaf Extract

Small finely pieces of 5 g of dried holy basil leaf sample was accurately weighed and placed into Erlenmeyer flask or beaker. Double distilled water (100 mL) was poured into the flask. The flask was then boiled for 5 min. The watery extract was cooled, filtered and kept in the refrigerator at 4 °C for further experiments and used within a week.

Preparation of 1 mM $AgNO_3$ Solution

Silver nitrate solution was prepared by adding 0.0169 g of $AgNO_3$ to 100 mL of deionized water in the Erlenmeyer flask and stored for further experiments.

Synthesis of Colloidal Solution of Silver Nanoparticles

Colloidal solution of silver nanoparticles were synthesized in accordance with the following procedure.

Different volumes (5, 10, 15, 20 and 25 μL) of Holy basil leaf extract were typically added to 2, 4, 6, 8 and 10 mL of silver nitrate solution (0.001M) respectively to be reduced Ag^+ ion to Ag^0 (nanoparticles). The colloidal solution of silver nanoparticles colouring colloidal brown was obtained within an hour (for about 50 min). The solution was allowed to incubate at room temperature overnight. The colloidal solution obtained were stored in refrigerator until further use to analyse.

Confirmation for The Existence of Silver Nanoparticles in Solution by Tyndall Effect

A laser pointer was taken and wrapped a rubber band around the on-switch so it was on continuously. The laser pointer was placed to the edge of the bottles containing GNP colloidal solution and the light was passed through the solution. The observation was recorded. The laser pointer was then rotated at 90° intervals and recorded any new observations.

The photograph of observation about the existence of silver nanoparticles in solution are represented in Figure 1.

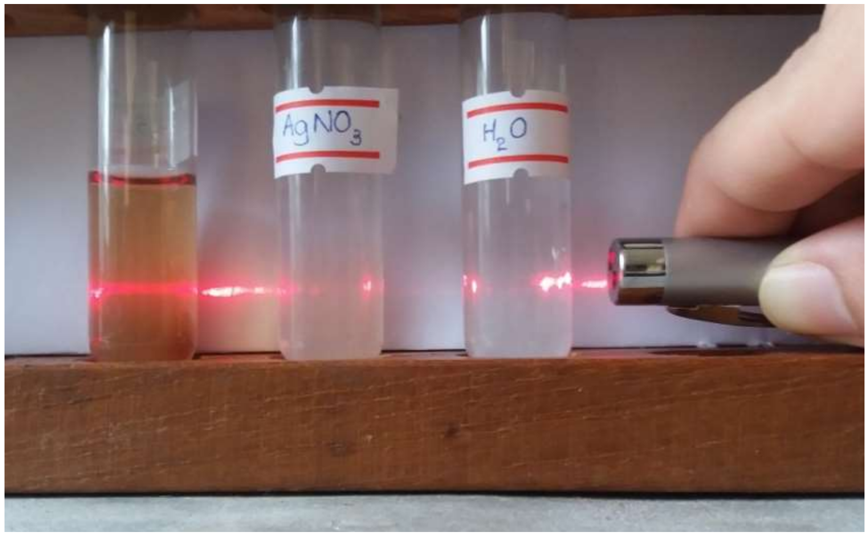

Figure 1: Dispersion of light (by laser beam) through AgNPs in aqueous medium

Confirmation the Formation and the Presence of Silver Nanoparticles (a Colloidal Solution) by the Spectra of the UV-Visible Spectrophotometry

The sample solutions were first diluted with distilled water. The UV-visible spectroscopic measurements of holy basil leaf extract, AgNP-8 and AgNP-10 were carried out by a computer controlled on a UV-visible Spectrophotometer (Shimadzu) at the West Yangon University.

Confirmation of the Functional Groups of Silver Nanoparticles by FTIR Spectrophotometry

The characterization of functional groups on the surface of AgNPs by plant extract were investigated by FTIR analysis (Shimadzu) and the spectra was scanned in the range of 4000-400 cm^{-1} range. The sample were prepared by dispersing the AgNPs uniformly in a matrix of dry KBr, compressed to form an almost transparent disc. KBr was used as a standard analyse the samples.

Determination of Crystallite Size Using Scheerer's Formula and Identification for Structure of Prepared Silver Nanoparticles from XRD Diffractograms

The synthesized colloidal AgNP solution was incubated at room temperature for least 2 days. The next day, the solution was observed to have distinctly deposited precipitate at the bottom of flask, leaving the colloidal supernatant at the top. The upper aliquot (colloidal AgNP) was decanted stored in refrigerator for further use. The precipitated silver nanoparticle powder was obtained from 5 mL of silver nitrate solution (0.001 M) and it was designated as AgNP-2 and the others as AgNP-4, AgNP-6, AgNP-8 and AgNP-10 respectively according to their concentrations.

The samples of silver nanoparticle powder (AgNP-2, AgNP-4, AgNP-6, AgNP-8 and AgNP-10) were analysed by X-ray diffraction technique to identify the crystallite size and structure. The recommended procedure used was in accordance with the catalogue.

The XRD spectra of the sample are presented in Figures 2 to 6. The average crystallite size of AgNP samples was calculated by using Scherrer's formula.

$$L = \frac{K\lambda}{\beta \cos\theta}$$

where, L = average crystallite size

K = constant crystallite shape

λ = wavelength of X-ray radiation $CuK\alpha_1$

β = FWHM of 2θ

θ = diffraction Bragg angle

Tables 1 and 2 show the average cryatsllite size of prepared silver nanoparticles.

Results and Discussion

Existence of Silver Nanoparticles in Solution by Tyndall Effect

The Tyndall effect is the scattering of light as a light beam passes through the colloid. Figure 1 shows the existence of silver nanoparticles in aqueous solution (as colloids). This Tyndall effect could be evident that the colloids have dispersed particles, making them nanoparticles. Hence, the particles will not settle out of the mixture.

According to this evidence, it was found that laser beam does not pass through water and silver nitrate solution. The dispersion of light passes through AgNPs in aqueous medium.

Confirmation to Colloidal AgNP-8 and AgNP-10 in Aqueous Solution by UV-Visible Spectrophotometry

UV-visible spectroscopy is a valuable tool for structural characterization of silver nanoparticles.

Figures 2 to 4 show the UV absorption spectra of colloidal AgNP-8, AgNP-10 and watery extract of holy basil leaf, respectively. It indicated that the silver nanoparticles were highly formed in aqueous phase, being stable with no precipitation. The maximum UV-visible absorption peak of colloidal AgNP-8 and AgNP-10 were appeared at 415 nm and 424 nm whereas watery extract of holy basil leaf absorbed the UV light at 268 nm.

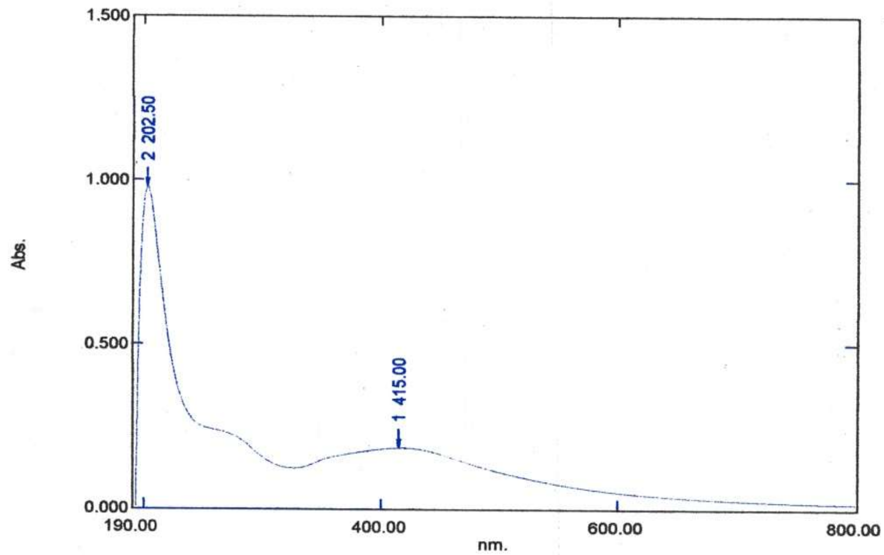

Figure 2: UV spectrum of colloidal AgNP-8

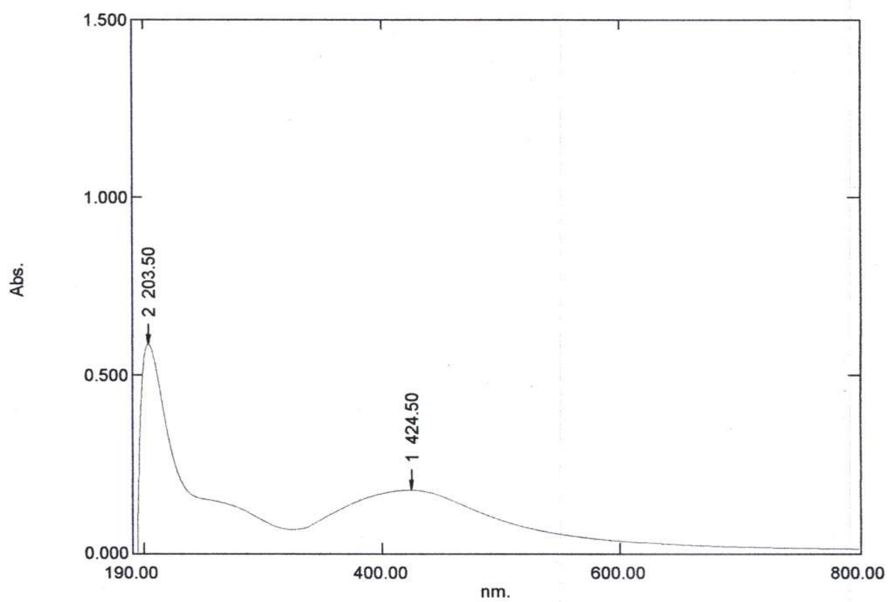

Figure 3: UV spectrum of colloidal AgNP-10

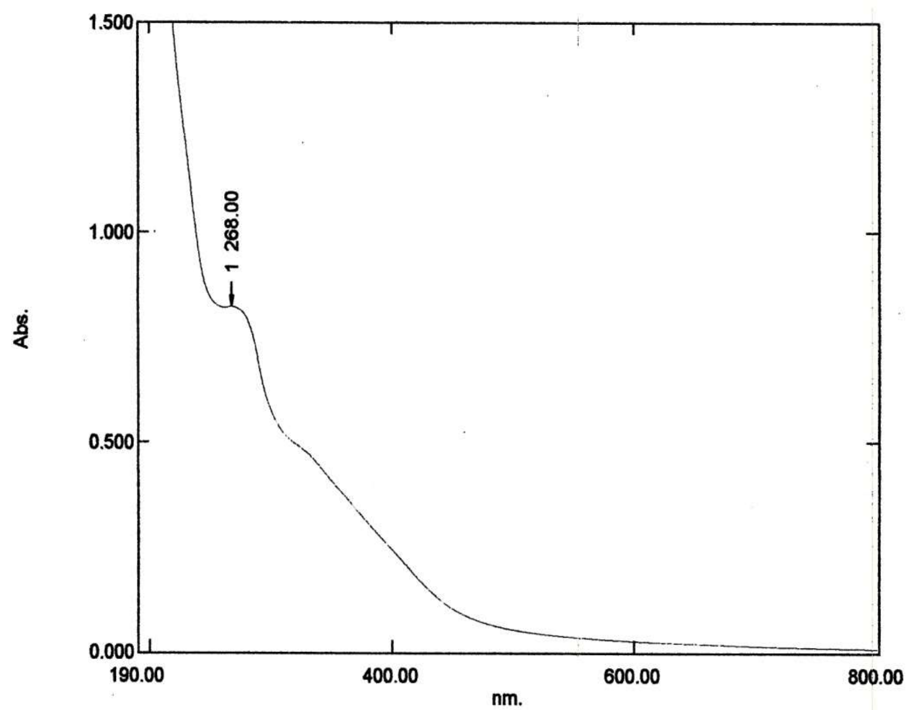

Figure 4: UV spectrum of watery extract of holy basil leaf

Confirmation of the Functional Groups of Silver Nanoparticles by FTIR Spectrophotometry

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectra of silver nanoparticles show figures 5 and 6 and the bands at 3348 cm^{-1} and 3361 cm^{-1} corresponds to O-H stretching of alcohols and phenols. The peak found that 1636 cm^{-1} and 1647 cm^{-1} showed C=O stretching of conjugated system.

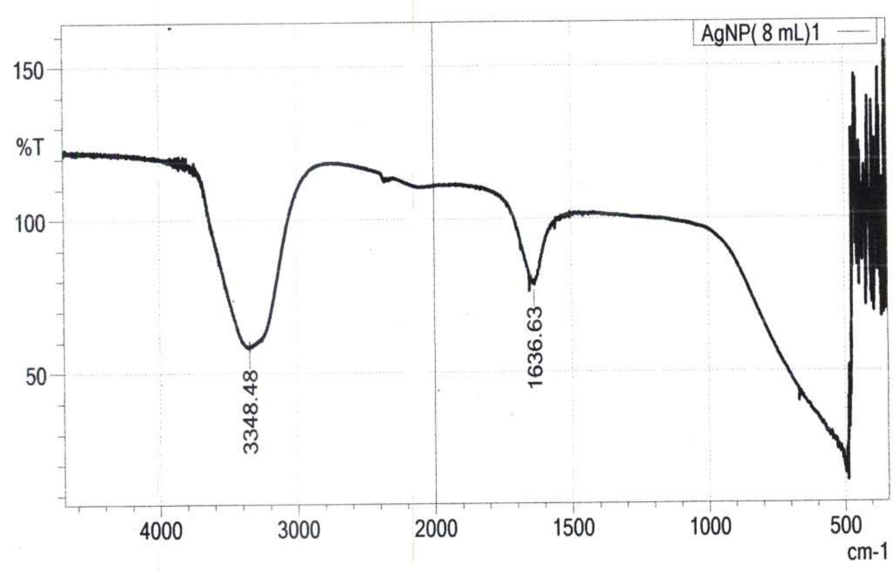

Figure 5: FTIR spectrum of colloidal AgNP-8

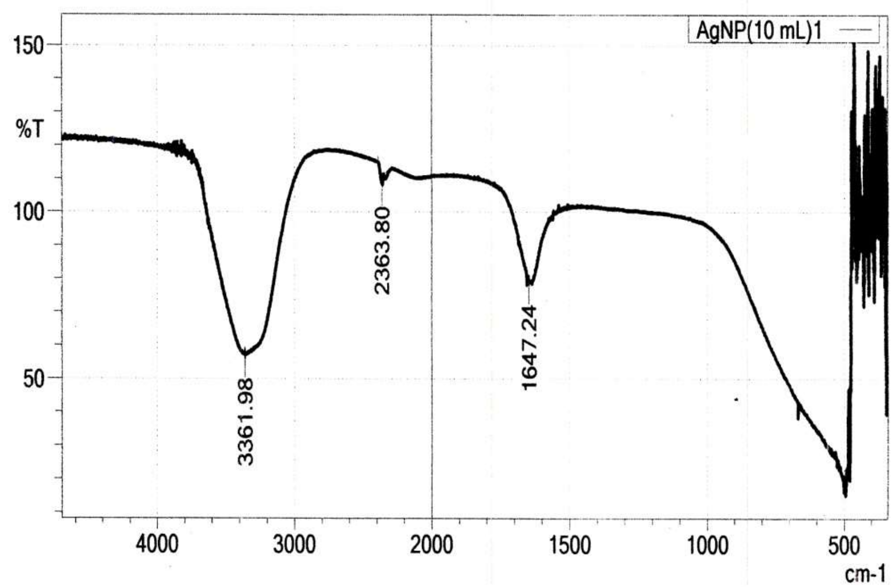

Figure 6: FTIR spectrum of colloidal AgNP-10

Structure of Prepared Silver Nanoparticles and the Crystallite Size by Using Scheerer's Formula from X-Ray Diffractograms

Figures 7 to 11 represent the X-ray diffractograms of the AgNP and Tables 1 and 2 describe the average crystallite size with respect to their relating parameters of 2θ, d and FWHM (the full-width at half maximum). The Bragg reflections corresponding to the (1 1 1), (2 0 0) and (2 2 0) sets of lattice planes were observed from the patterns.

These Miller indices corresponding to the (1 1 1), (2 0 0) and (2 2 0) all-odd or all-even sets of lattice planes designate the face-centred cubic (FCC) structure of silver nanoparticle.

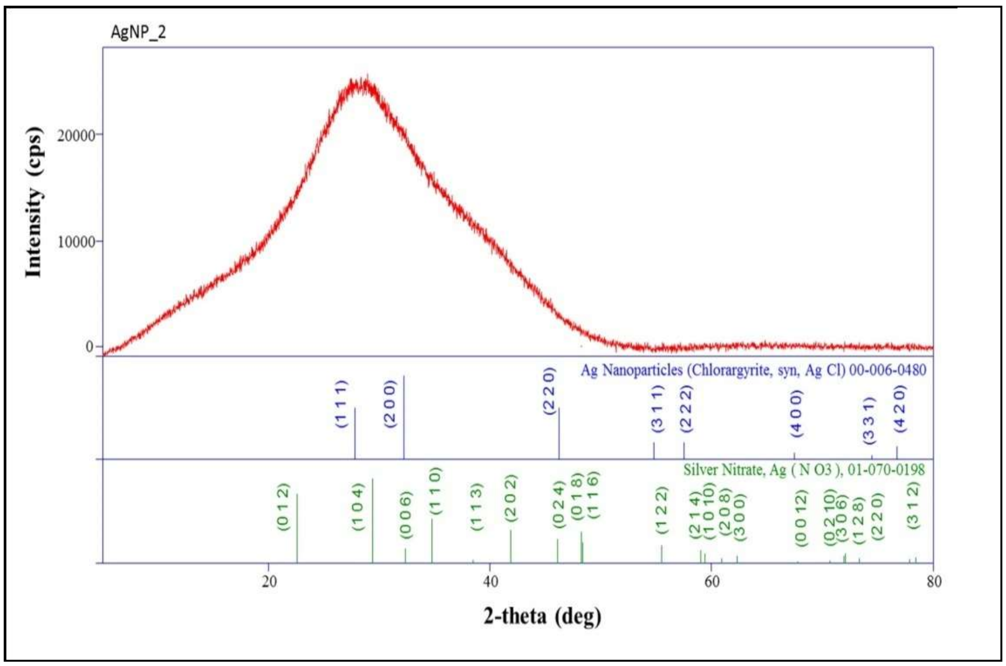

Figure 7: X-ray diffractogram of AgNP-2

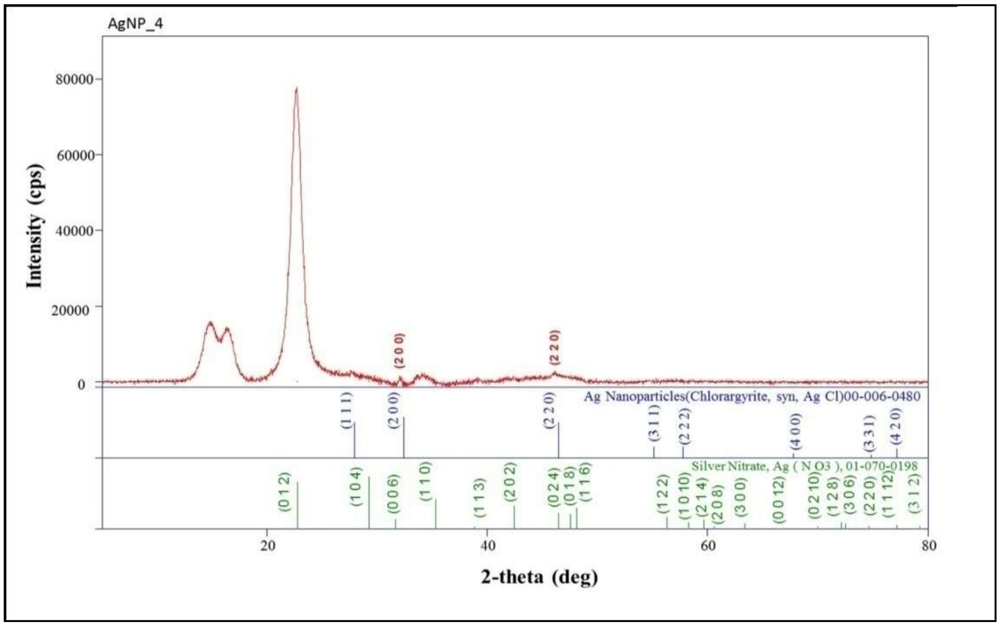

Figure 8: X-ray diffractogram of AgNP-4

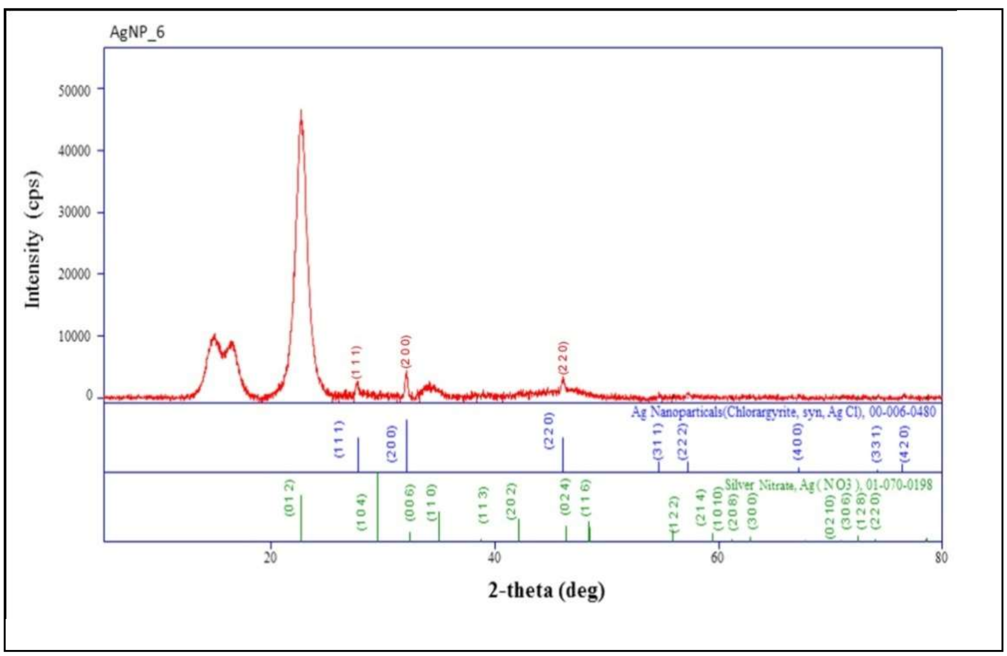

Figure 9: X-ray diffractogram of AgNP-6

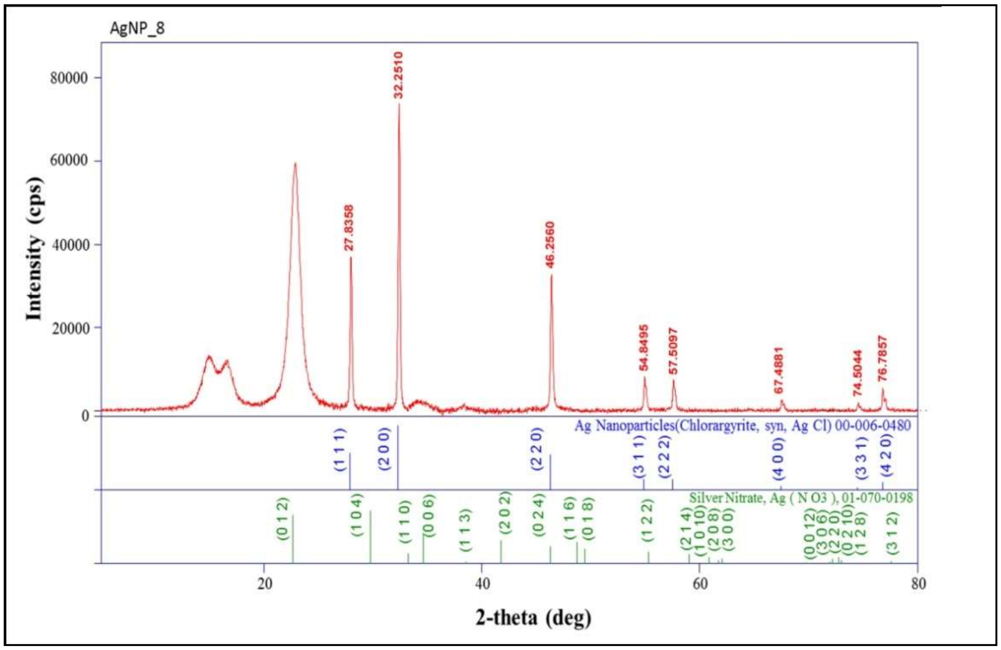

Figure 10: X-ray diffractogram of AgNP-8

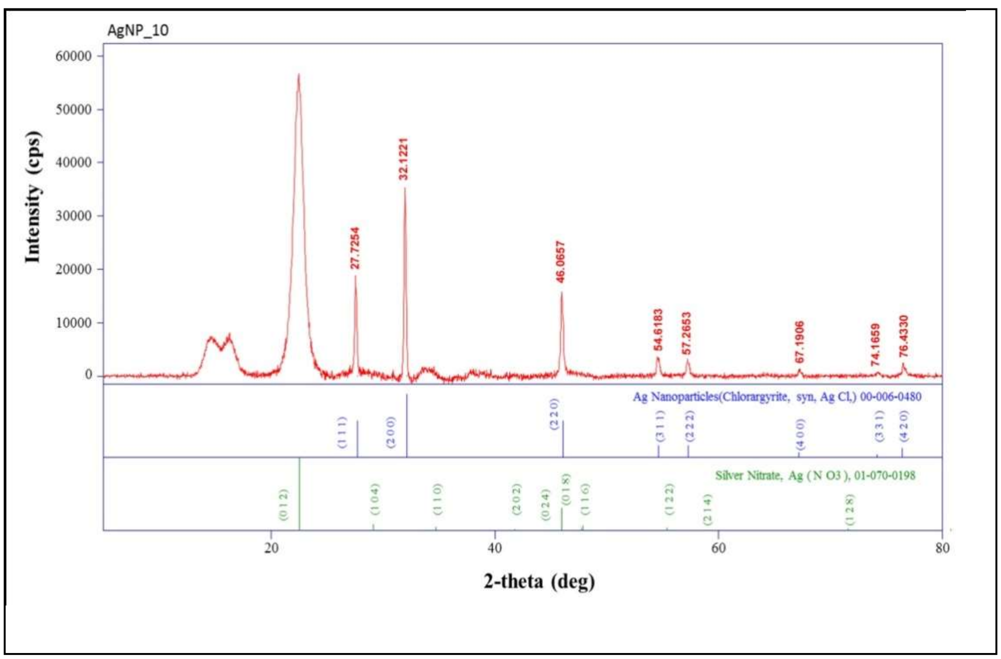

Figure 11: X-ray diffractogram of AgNP-10

Table 1: Data for Calculation of Average Crystallite Size of AgNP-8

Peak	2θ (deg)	θ (deg)	cosθ (rad)	β of FWHM (deg)	β (rad)	L (nm)
1	27.8358	13.9179	0.9706	0.1634	0.002852	50.09
2	32.2510	16.1255	0.9607	0.1618	0.002824	51.11
3	46.2560	23.1280	0.9196	0.1577	0.002752	54.78
4	54.8495	27.4248	0.8876	0.1561	0.002724	57.34
5	57.5097	28.7549	0.8767	0.1558	0.002719	58.16
6	67.4881	33.7441	0.8315	0.1556	0.002716	61.40
7	74.5044	37.2522	0.7960	0.1565	0.002731	63.77
8	76.7854	38.3927	0.7838	0.1571	0.002742	64.52
				Average crystallite size, L =		57.65

Table 2: Data for Calculation of Average Crystallite Size of AgNP-10

Peak	2θ (deg)	θ (deg)	cosθ (rad)	Bof FWHM (deg)	β (rad)	L (nm)
1	27.7254	13.8627	0.9709	0.1860	0.003246	43.99
2	32.1221	16.0611	0.9610	0.1855	0.003238	44.57
3	46.0657	23.0329	0.9203	0.1838	0.003208	46.97
4	54.6183	27.3092	0.8885	0.1827	0.003189	48.94
5	57.2653	28.6327	0.8777	0.1824	0.003183	49.62
6	67.1906	33.5953	0.8330	0.1809	0.003157	52.72
7	74.1659	37.0830	0.7978	0.1798	0.003138	55.38
8	76.4330	38.2165	0.7857	0.1794	0.003131	56.36
				Average crystallite size, L =		49.82

Conclusion

Ocimum sanctum L. (holy basil) leaves having positive and sound benefits have to be used in synthesis of silver nanoparticles. The UV absorption peaks of AgNP-8 and AgNP-10 were found to be 414 nm and 425 nm indicate the synthesis of AgNPs. FTIR confirmed the biofabrication of the AgNPs by the action of different phytochemicals with its different groups present in the extract solution. From the X-ray diffraction characterization, it was observed that the XRD patterns of AgNP-8 and AgNP-10 from synthesized AgNPs only showed the optimal crystallite aggregates for cubic crystal system. Comparing with (JCPDS, DB card No. 00-006-0480) reference, the peaks at 2θ values of both AgNP-8 and AgNP-10 corresponded to the Miller indices ($h\ k\ l$) of cubic system of Ag nanoparticles. From indexing XRD patterns of cubic system, it could be confirmed that the optimal phyto-synthesized AgNP-8 and AgNP-10 occupied by face-centered cubic (FCC) crystal system because of having Miller indices with all odd or all even. It was observed that the average crystallite sizes of AgNP-8 and AgNP-10 were found to be 57.65 nm and 49.82 nm by using Scherrer formula.

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