

Moss (Bryophyte) Mediated Synthesis and Characterization of Silver Nanoparticles from *Campylopus flexuosus* (Hedw.) Bird.

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Abstract:

This study aims to synthesize and characterize the silver nanoparticles from *Campylopus flexuosus* (Hedw.) Bird. This is achieved through 90ml 1mM AgNO₃ mixed with the 10ml of plant extract. Occurrence of visible colour change of reddish brown indicates the formation of AgNps. The AgNps were characterized based on UV-Vis, FTIR, FESEM, Zeta potential, particle size and XRD analysis. The maximum absorption obtained at 436nm in UV-Vis spectroscopy. Presence of Carbonyl compounds were acted as a capping agent to the production of silver nanoparticles identified by the FTIR. FESEM and XRD analysis revealed the average size of the nanoparticles as 58nm. -25mv obtained in zeta potential showed a good stability.

Key words: *Campylopus flexuosus*, silver nanoparticles, UV-Vis, FTIR, FESEM, XRD

INTRODUCTION:

Nanotechnology is now a most inspired and highly fascinated science among the researchers. Nanotechnology embraces many fields like engineering, electronics, physics, material science, environmental remediation, cosmetic industries, agriculture and in life science especially biomedical field to manufacture the devices and drug delivery etc [1]. There are multiple methods applied to develop the synthesis of nanoparticles, such as physical, chemical, biological, microwave assisted, radiation assisted, electrochemical and sonochemical methods [2]. Compare to other methods, biological method is ecofriendly and no hazardous chemicals were involved. The green synthesis is most advantageous over the chemical and physical methods [3]. The metal nanoparticle synthesis carried out by silver, gold, platinum, zinc and copper [4]. Among these silver metal has been widely applied to the green synthesis process. This is due to the huge application in the medical field especially as an antimicrobial agent [5] and also an anticancer agent [6]. Most of the biosynthesis of silver nanoparticles was done by using higher plants like angiosperms, gymnosperms and pteridophytes. Fewer attempts were made in the lower cryptogamic plants like *Anthoceros*, *Riccia* [7] and *Fissidens* [8]. Hence the moss plant *Campylopus flexuosus* (Hedw.) Bird. has chosen to synthesize silver nanoparticles for the present study. Bryophytes are primitive, nonvascular land plants and show the simple organization of the thalloid plant body. Among the bryophytes mosses were the advanced group occupied the unique position between lower cryptogams and vascular cryptogams [9]. *C. flexuosus* is an acrocorpous moss, belongs to the order Dicranales, family Dicranaceae. It is Dioicous, somewhat shiny, green to olive-green, plants in dense tufts, variable size and vigour, 1-10cm.High, tomentose, dichotomously branched. Leaves erect to erectopotent, flexuose, when dry \pm 6mm long, lanceolate-subulate, from a wider base slowly

narrowing down into a canaliculate subula with incurved margins. Leaf tip margin serrate and the tip not hyaline. Costa about $\frac{1}{2}$ the leaf base, with substereide bundles on the dorsal side but the dorsal surface smooth. Alar cells brown, highly bulging, rather large formed of large inflated, lamina cells at base long rectangular, near costa becoming about half as narrow near margin, almost rhomboidal and incrassate at margin [10].

MATERIALS AND METHODS

Collection and extraction of plant material

The plant *C. flexuosus* was collected from Kuzhivalavu of Kolli Hills, Eastern Ghats from Namakkal District, Tamilnadu, India. The collected mosses were shade dried and made it into powder. 10gm of powder taken in 250 ml Erlenmeyer flask along with 100 ml of distilled water and boiled the mixture for 30 min. The extract was filtered through the whatmann No.1 filter paper and stored in the refrigerator for further use.

Synthesis of silver nanoparticles

To synthesize the silver nanoparticles 10ml of plant extract were mixed with 90ml of aqueous 1mM AgNO₃ solution in a 250 ml Erlenmeyer flask. The flask was exposed to sun light for an hour.

Characterization of synthesized silver nanoparticles

The reduction of biosynthesized silver nanoparticles was subjected to (perkin-Elmer Lamda 35) UV-Visible spectroscopy in the range of 200nm-800. FTIR spectra were obtained using a spectrophotometer (Spectrum RX 1, Perkin Elmer) in the spectral region of 4000cm⁻¹- 400cm⁻¹. The morphology, size and Elemental analysis of the prepared silver nanoparticles was examined using FESEM with EDX (Carl Zeiss, SUPRA 55 model). The distribution pattern of AgNps particle size were evaluated by Dynamic light scattering analysis (DLS) and the stability were determined by the Zeta potential analysis by zeta sizer (Malvern instrument Ltd, UK). Determination of the

crystallinity was done by XRD analysis with the advance diffractometer (Bruker Ax D8). XRD values had been calculated using Debye-Scherrer's formula $D = k \lambda / \beta \cdot \cos\theta$.

RESULTS

Confirmation of biosynthesized AgNps

Present study is to focus on the green synthesis of silver nanoparticles using *C. flexuosus* employed with silver nitrate solution. Silver nanoparticle formation confirmed by the pale yellow colour change into reddish brown colour (Figure 1). Indication of colour change is the primary confirmation of nanoparticle synthesis. Further these synthesized silver nanoparticles were confirmed through the characterization studies like UV-Visible spectroscopy, FTIR, FESEM, EDAX, Zeta potential, Particle size and XRD.

UV-Visible spectral analysis

Colour change confirmed the reduction of Ag ions due to the excitation of surface resonance. Colloidal solution of silver nanoparticles subjected to UV-Vis spectral analysis where observed the maximum absorbance at 436nm (Figure 2).

FTIR (Fourier transform infrared) analysis

Fourier transform infrared (FTIR) analysis was performed to identify the possible biomolecules responsible for the

reduction of the silver ions and capping of the Ag-NPs. The spectrum (Figure 3a) of dried aqueous extract of *C. flexuosus* showed the peak at 3405cm^{-1} corresponds to the N-H stretch of amine group. The peaks in the regions of $2128, 2855$ and 2925cm^{-1} were assigned to C-H and C=C stretching is the vibration of aldehyde and alkyne respectively. The peak observed in 1729 represent the C=O stretching of aldehyde, $1633, 1430, 1384, 1318\text{cm}^{-1}$ were assigned to C=C, O=H and C-N stretching and bending vibration of alkane, carboxylic acid, aromatic amine respectively. $1251, 1203, 1157, 1059\text{cm}^{-1}$ peaks correspond to C-H, C-O, S=O stretching is the vibration of alkane, aromatic esters, primary alcohols and sulfonic acid. The peaks $780, 669\text{cm}^{-1}$ were assigned to C=C bending of alkanes. The spectrum (figure 3b) of the synthesized CfAgNps (*C. flexuosus* silver nanoparticles) showed the peaks at 825 and 876cm^{-1} were assigned to C-H aromatic stretch. 1040 and 1201cm^{-1} were assigned to C-O ether and alcoholic stretches. 1384 and 1631 corresponds to C=C and C-H stretch (alkene & alkane). 2853 and 2924 were assigned to C-H alkyl stretch. 3398 peak shows the N-H amine stretch. So the FTIR analysis reveal the carbonyl compounds of aldehyde and carboxylic acids were bind to the metal might be acted as the capping agent for the production silver nanoparticles.



Figure 1: A) plant extract (pale yellow) B) Silver Nitrate C) plant extract + silver nitrate colour change (Reddish brown)

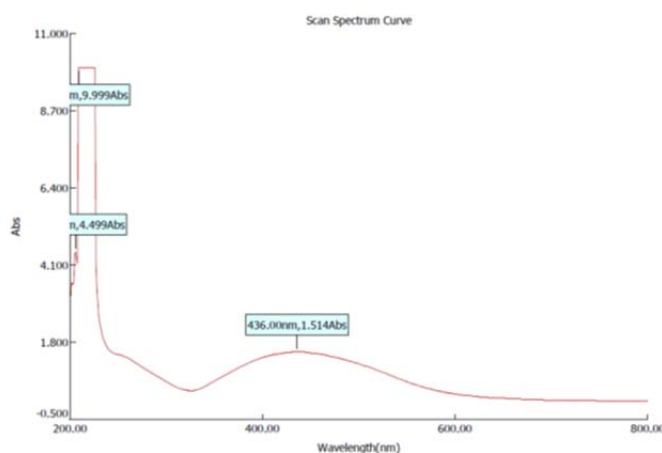
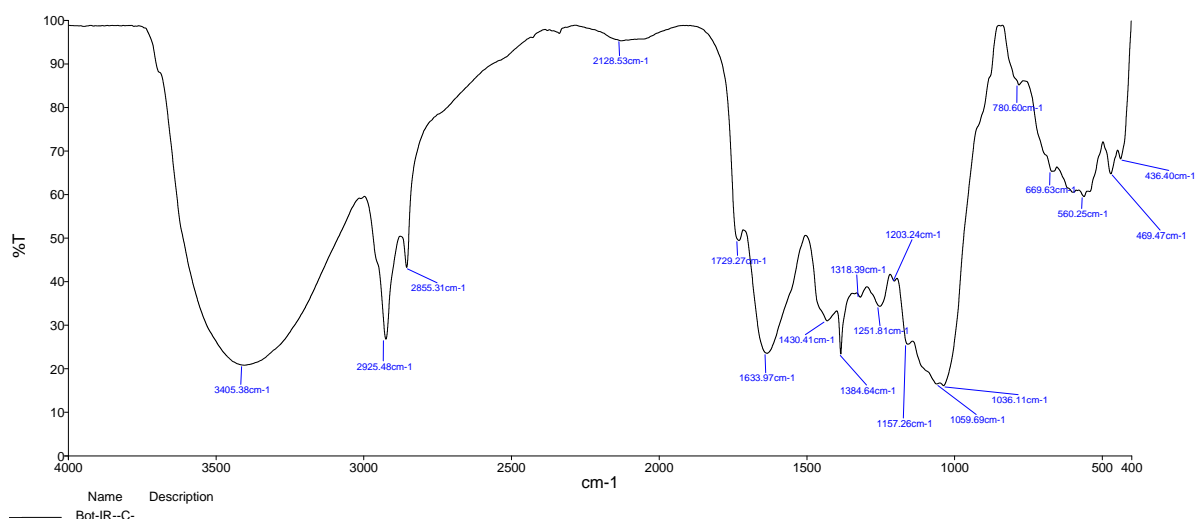
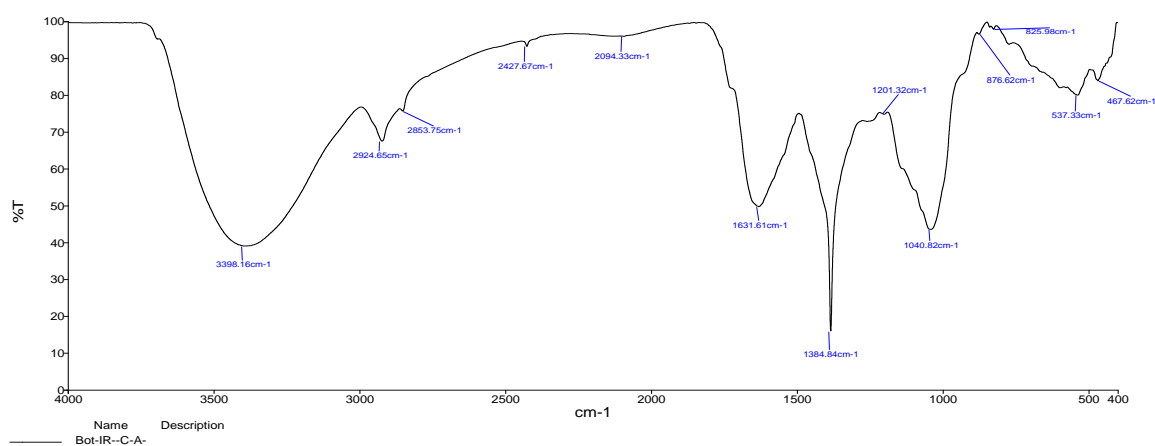


Figure 2: UV- Visible spectrum for *Campylopus flexuosus* mediated synthesized silver nanoparticles

Figure 3(a): FTIR peaks for *Campylopus flexuosus* plant extractFigure 3(b): FTIR peaks for *Campylopus flexuosus* mediated synthesized silver nanoparticles

FESEM (Field Emission Scanning Electron Microscopy) analysis

The size of the biosynthesized silver nanoparticles was determined by the Field emission scanning electron microscopy (Figure 4). FESEM image revealed the size of the CfAgNps mediated synthesized silver nanoparticles. The particle size between the 50nm-70nm.

EDAX (Energy Dispersive Analysis of X Ray) analysis

EDAX is carried out to determine the quantity of elements present in the CfAgNps. EDAX image (figure 5) showed the strong signal peaks were obtained in the 3kev due to the surface plasmon resonance and 4.98% quantity of silver detected. It indicates the presence of silver ions in the synthesized silver nanoparticles.

Zeta Potential analysis

The zeta potential analysis used to determine the nanoparticle surface stability, dispersion and electrophoretic mobility of the synthesized silver nanoparticles. The zeta potential value for the CfAgNps is -25mv the negatively charged particles confirmed the repulsion among the particles so thereby negative values ratify the strong stability of the synthesized silver nanoparticles (figure 6).

Particles size analysis

The particle sizes were determined by the dynamic light scattering method (Figure 7). It showed the particles size distribution of the synthesized silver nanoparticles. Distribution of the particle size for the synthesized AgNps revealed the various sizes of the particles observed from 28.04nm to 180nm. Precisely the average particles size for CfAgNps is 113nm.

XRD (X Ray diffraction) analysis

This is another confirmation analysis of the size and crystallinity of the synthesized silver nanoparticles. XRD analysis (figure 8) confirmed the crystallinity and (Table 1) size of CfAgNps. Debye – Scherrer's formula is commonly used to determine the crystallinity of the nanoparticles. $D = \frac{k \lambda}{\beta \cos \theta}$. where, D – Average crystalline size (nm), k – Dimensionless shape factor (0.9), λ – X ray wavelength (0.1541 nm), β – Angular / line broadening at FWHM of the XRD peak at the diffraction angle, θ – Diffraction angle (Table1). The peaks obtained in $2\theta = 37.80^\circ, 43.96^\circ, 64.18^\circ, 77.13^\circ, 31.917^\circ$. So the XRD pattern clearly exhibit the average size of the particle is 51nm and the crystallinity is face centered cubic structure.

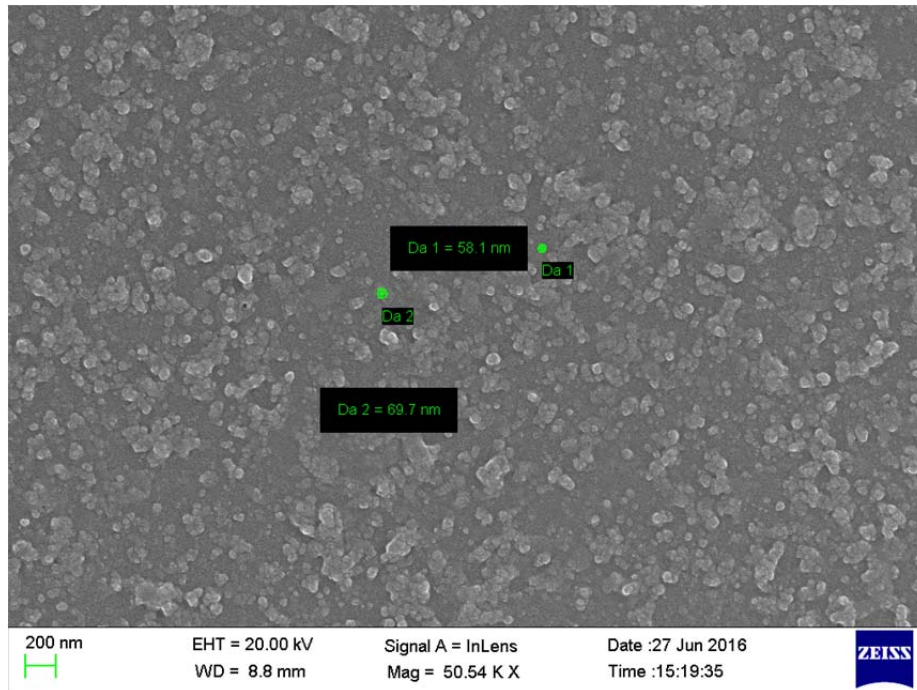


Figure 4: FESEM micrograph of AgNps synthesized from *Campylopus flexuosus* extract

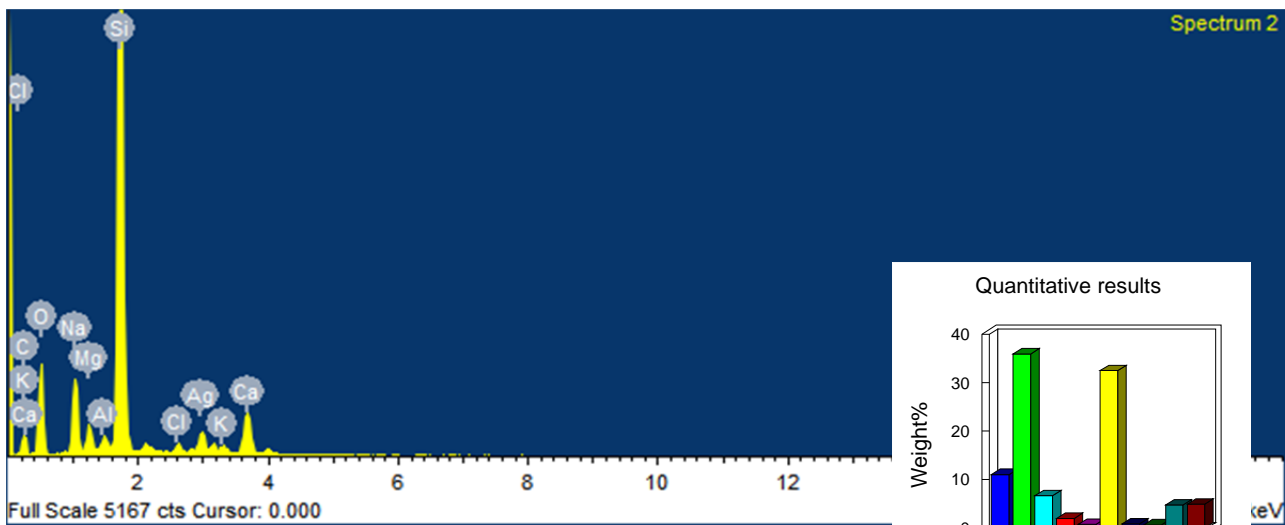


Figure5: EDAX spectral and quantitative analysis of silver nanoparticles synthesized from *Campylopus flexuosus* extract

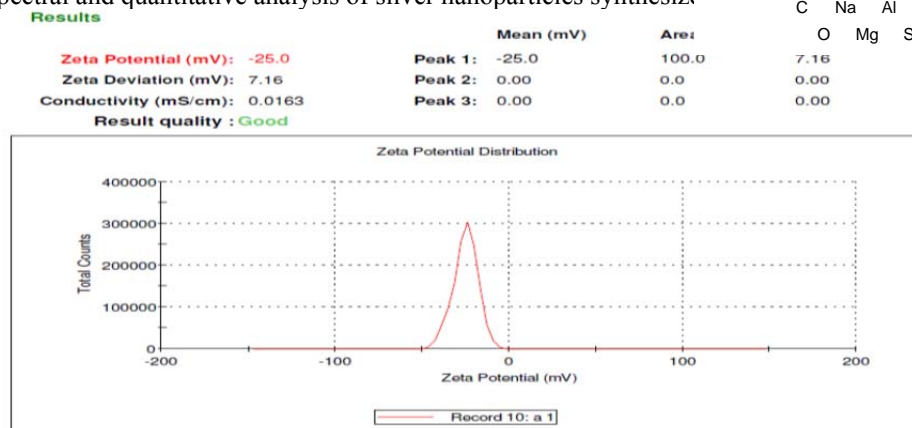


Figure 6: Zeta potential analysis of the silver nanoparticles synthesized from *Campylopus flexuosus* extract

Results

Z-Average (d.nm): 113.0	Peak 1: 180.1	Size (d.nm): 180.1	% Intensity: 95.0	Width (d.nm): 98.56
Pdl: 0.395	Peak 2: 28.04	28.04	5.0	6.479
Intercept: 0.881	Peak 3: 0.000	0.000	0.0	0.000
Result quality : Good				

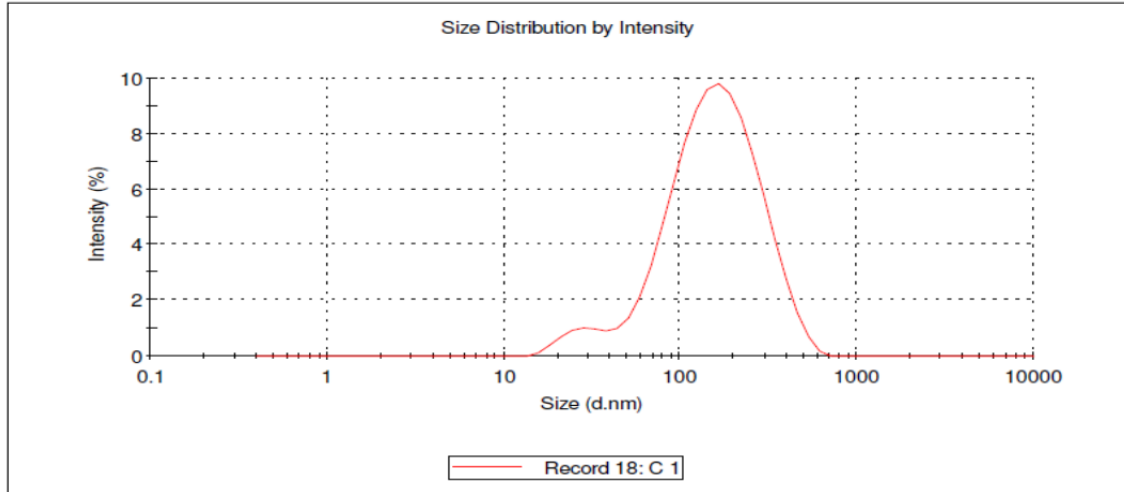


Figure 7: Particle size analysis of the *Campylopus flexuosus* mediated synthesized silver nanoparticles

C(NPS)

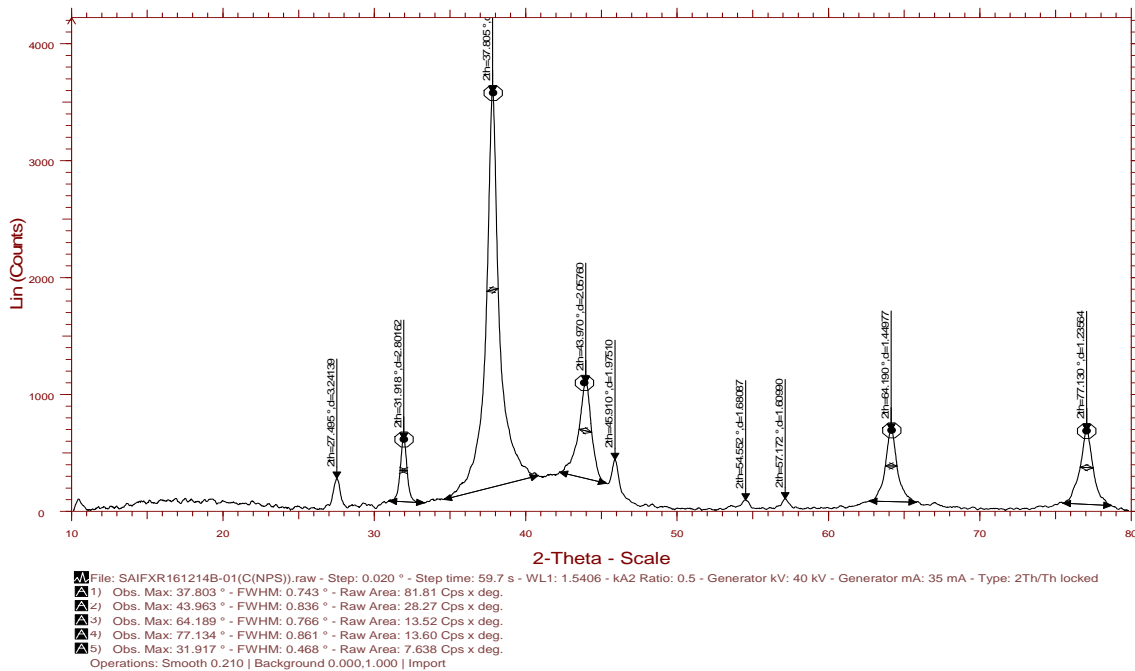


Figure 8: XRD analysis silver nanoparticles synthesized from *Campylopus flexuosus*

Pos. [°2Th.]	FWHM Left [°2Th.]	d-spacing [Å]	Particle size
37.803	0.743	2.37813	11.8
43.963	0.836	2.05760	10.7
64.189	0.766	1.44977	12.7
77.134	0.861	1.23564	12.3
31.917	0.468	2.80162	18.4

Table 1: Determination of crystalline size of AgNP's by using Debye-Scherrer's equation

DISCUSSION

Currently many approaches have been used to synthesize the silver nanoparticles in simple and easy methods. It includes both chemical and biological methods. Nowadays the nanoparticle synthesize using plants were attracted by many researchers (Kesarala Mohan Kumar, 2012)[11]. Thereby the greener synthesis process is (i.e synthesis of silver nanoparticles using plants) is easy and eco friendly method and also cost effective (Abduz Zahir A et.al., 2012)[12]. In the present study the moss (Bryophyte) plant is mediated to synthesize the silver nanoparticles. Addition of silver nitrate with the plant extract exhibit a colour change of yellow to reddish brown with a particular duration of incubation time. This is the preliminary confirmation of silver nanoparticle. The same result was obtained in the moss plant *Fissidens minutus* (Srivastava A.A et.al., 2011) [8]. Silver nitrate dissolved in water turned into silver free ions Ag⁺ to Ag⁰. By adding the plant extract to silver nitrate, the free silver ions gets the electron moiety and it will form into elemental silver (Fu M et.al., 2006) [13]. Formation of reddish brown colour is due to the surface plasmon resonance (Sathyavathi R et.al., 2010) [14]. The absorption maxima in UV-Vis is at 436nm, very similar result were obtained in *Anthoceros* mediated AgNps where λ max is at 438nm (Kulkarni A.P et.al., 2012) [7]. FTIR analysis revealed the presence of carbonyl compounds acted as a capping agent to synthesize the AgNps. The same result were reported in the aqueous extract of *Amaranthus dubius* AgNps (Jannathul Firdhouse et.al., 2012)[15]. FESEM analysis showed the 50 to 70nm in the CfAgNps. The result were accordance with *Bacillus* AgNps (Vithiya K et.al., 2014) [16]. Elemental analysis showed the quantity of silver is followed by C, Cl, O and Si the result obtained by (Ibrahim HMM, 2015)[17] The zeta potential value showed for CFAgNps the negative repulsion the similar result were corroborated with *Urtica dioica* synthesized AgNps (Kumari Jyoti 2016) [18]. Particle size distribution revealed the 113nm average size particles the similar results were obtained in the *Ficus carica* (Hemant P et.al., 2013) [19]. XRD pattern determined the average particle size is 51nm same results were matched with *Argemone mexicana* AgNps 20nm size ranges from 10 to 50nm (Singh A et al., 2010)[20].

CONCLUSION:

The plant mediated synthesized metallic nanoparticles have a high impact in the field of bio nanotechnology, but work in green synthesis by bryophytes is very meagre. Hence the present investigation aims on synthesis and characterization of silver nanoparticles from the moss (bryophyte) plant *Campylopus flexuosus*. Colour change is the primary confirmation of silver nanoparticle formation. Characterization studies were carried out by UV-Vis, FTIR, FESEM, EDAX, Zeta potential, Particle size and XRD.

Maximum absorption in UV-Vis is at 436nm confirmed the synthesis. Carbonyl compounds of protein binds to the surface of the metal so these act as a capping agent confirmed by the FTIR spectrum. Average particle is 50-70nm is determined by FESEM and XRD analysis. Overall results clearly exhibited the nanoparticles was successfully synthesized. Thus the present investigation proved that the moss plant *Campylopus flexuosus* moss plant has the potential to fabricate the nanoparticles.

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