

BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *ACTINOPTERIS RADIATA*, BEDD. AND ASSESSMENT OF THEIR ANTIMICROBIAL ACTIVITY

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Abstract

Introduction: *Actinopteris radiata*, Bedd. (Pteridaceae) is a true fern listed among endangered, rapidly fading plant species explored negligibly for its medicinal and phytochemical constituents. In light of the fact, the present investigation was focused to synthesise silver nanoparticles (AgNPs) from aqueous leaf extracts of *Actinopteris radiata* via plant models. **Materials and methods:** Crude leaf extracts were screened for different phytochemical and free radical scavenging assays. Moreover, the green synthesised silver nanoparticles were characterized by UV- Vis Spectrophotometer, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray spectroscopic (EDX) analysis respectively. Similarly, the antimicrobial efficacies of synthesized nanostructures and its effects on pathogenic microorganisms were enumerated. **Results and Discussion:** Phytochemical screening revealed substantial amount of bioactive entities *viz*; flavonoids, cardiac glycosides, tannins *etc.* and the synthesized silver nanoparticles were in the range of 200-800 nm. The X-ray diffraction pattern of the synthesized nanomaterials was found to be crystalline in nature at around 32nm. The SEM executed spherical shape with average particle size of 18.18 nm. Also, antimicrobial activity evidenced maximum growth kinetics against gram negative bacterial strains (*Klebsiella pneumonia* and *Penicillium digitalis*) than the gram positive organisms. **Conclusion:** Therefore, it can be suggested that the biosynthesized silver nanoparticles produced may enact as a capping agent. Furthermore, these nanoparticles are non-toxic in nature and therefore could be used for the preparation of herbal drug formulations so as to alleviate various diseases.

Keywords: *Actinopteris radiata*, green synthesis, spectroscopy, antimicrobial.

INTRODUCTION

In recent centuries, nanotechnology is an emerging field of research for the pioneers in science. It allocates with nanomaterials having the size ranged between 1-100 nm^[1] and they display a unique property in their size and dispersal. Biologically, synthesis of metal nanoparticles are mass-produced using plant extracts and they are deliberated as the most widely adopted method of green, eco-friendly production of nanoparticles since the plants are

abundant in distribution, easily available, much safer to handle and act as a cradle of numerous metabolites. Nanocrystalline silver units can act as antiviral agents, photosensitizers, anticancer therapeutic agents in the treatment of various cancers.^[2] Silver is renowned for processing inhibitory effect pertaining to several bacterial strains and microbes frequently exists in medical and industrial activities.^[3]

Nanoparticles can be synthesised from a varied biological entities such as bacteria, virus, algae, fungi, yeast and plants. Each biological entity has varying degrees of biochemical dispensation capabilities can be effectually expended to synthesis specific metallic or non-metallic oxide nanoparticles.^[4] Not all biological entities can synthesis nanoparticles owing to their enzyme actions and inherent metabolic process. Consequently, assortment of appropriate biological entities is indispensable to yield nanostructures with distinct properties such as size and morphology. Therefore recently, the biosynthesis of nanomaterials using plant and plant excerpts seems to be the striking substitute to conventional chemical synthesis. Besides, amalgamations of molecules present in plant extracts accomplish as both reducing and stabilising (capping) proxies in the course of nanoparticle synthesis.

Actinopteris radiata, Bedd. (Pteridaceae) commonly known as peacock tail, is a true fern listed among endangered and rapidly fading plant species, explored negligibly for its medicinal and phytochemical constituents. It is used widely as a dietary supplement for malnutrition and a common remedy to treat headache, cough, blood pressure, tuberculosis, respiratory and cardiovascular diseases.^[5] This plant has been reported to possess innumerable biological activities such as antioxidant, anti-diabetic, antimicrobial activity^[6], antifertility^[7], anthelmintic^[8], anti-stress and anti-allergic effect on asthma.^[9] Considering the immense potentiality of *A. radiata* as biological source, the contemporary study was addressed to characterize the synthesized silver nanoparticles using UV-Vis Spectroscopy, Scanning Electron Microscope (SEM), Energy Dispersive X-Ray (EDX) and X-Ray diffraction (XRD) analysis and also to gauge the free radical quenching efficacies, antimicrobial activity using plant extracts.

MATERIALS AND METHODS

Chemicals and Reagents

Silver nitrate (AgNO_3) was obtained from Hi Media Pvt. Ltd., Mumbai. All other reagents and solvents used were of analytical grade. The glasswares used were subjected to sterilization process before commencement of the experimental process.

Collection and Preparation of crude plant extracts

Fresh leaves of *A. radiata* were harvested from Kallar river basis of Mettupalayam, Tamilnadu, India. The collected plant samples were duly identified with the reference specimen preserved at Botanical Survey of India, Southern circle, Coimbatore. Fresh leaves of *A. radiata* were cleaned with plentiful amount of water to remove debris and other contaminated organic contents, followed by surface sterilization with double distilled water and shade dried at room temperature for 7 days. About 25gm of finely powdered leaves were kept in a beaker containing 250 mL of double distilled water and boiled for 30 min at 80°C. The extract was cooled, filtered with Whatman filter paper no.1 and the filtrate was used for the synthesis of silver nanoparticles.

Biosynthesis of Silver Nanoparticles (AgNPs)

Environmental free eco-friendly silver nanoparticles were prepared by green chemistry method using *A. radiata* leaf extract as a reducing agent. In brief, the 100mL of silver nitrate (AgNO_3) at the concentration of 1mM was prepared in Erlenmeyer flask, using a starting material. To obtain optimum concentration, 10mL prepared silver nitrate solution was taken in a series of five different test tubes. To this different concentration *viz.*, 1 mL, 2mL, 3 mL, 4mL and 5mL of prepared plant samples of *A. radiata* leaves were added respectively. Each reaction test tubes were placed in magnetic stirrer for 1h at 60°C for the green synthesis of silver nanoparticles. After the reaction time, change in colour was noted and photographed.

UV-Vis spectrophotometer analysis

After incubation of 24hours, all the samples were subjected to UV-Vis spectrophotometer analysis (JASCO V760, Japan) between 200 to 800nm for the confirmation of synthesized silver nanoparticles via green route method.

***In vitro* antioxidant activity**

DPPH radical Scavenging Activity

The antiradical efficiency was assessed by DPPH method as described by Blois (1958)^[10]. In this method commercially available methanol soluble, stable free radical DPPH was used. In its radical form, DPPH has an absorption band at 515 nm, which disappears upon reduction by an antioxidant compound or a radical species. For the photometric assay, 30 μL volume of synthesized silver nanoparticles solution (1, 2, 3, 4 and 5mL) were taken in different test tubes. The volume was adjusted to 100 μl with methanol. 5.0 ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand for 20 min at 27 °C. The control was prepared as above but without the

test extract was used for the baseline correction. Changes in the absorbance of the samples were monitored at 517 nm. The per cent of DPPH discolouration of the samples was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{517} \text{ of control} - A_{517} \text{ of sample}) / A_{517} \text{ of control}] \times 100.$$

Antioxidant activities of the extracts were expressed as IC₅₀, (the microgram of extract to scavenge 50% of the DPPH radicals) and were obtained by interpolation from linear regression analysis. A lower IC₅₀ value indicates greater antioxidant activity.

Trolox equivalent antioxidant capacity (TEAC) assay

Antioxidant activity was performed using an improved ABTS^{•+} method proposed by Siddhuraju and Manian (2007)^[11]. The ABTS radical cation (ABTS^{•+}) was generated by a reaction of 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate after incubation for 16 hrs at laboratory temperature in dark. Blue - green ABTS^{•+} was formed at the end of this period. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30°C to obtain an absorbance of 0.700 ± 0.02 at 734 nm, the wavelength of maximum absorbance in the visible region. The silver nanoparticles solution (1, 2, 3, 4 and 5 mL) in ethanol was diluted such that, after introduction of a 10 µL aliquot of each dilution into the assay, they produced between 20-80% inhibition of the blank absorbance. After the addition of 1.0 ml of diluted ABTS solution to 10 µL of silver nanoparticles solution or Trolox standards (final concentration 0-15 µM) in ethanol, absorbance (734 nm) was recorded at 30°C, exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicates were maintained for the experiments and the per cent inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration.^[12] The unit of Total Antioxidant Activity (TAA) was defined as the concentration of Trolox having the equivalent antioxidant activity expressed as µmol/ g sample extracts on dry weight basis.

Antimicrobial activity of silver nanoparticles

The antimicrobial potential of plant assisted synthesized silver nanoparticles were tested for their effect against the growth of pathogenic bacteria and fungus by disc diffusion method.^[13] The inoculums of test organisms were prepared by growing pure isolates in nutrient broth at 37°C for overnight. The agar plates were prepared by pour plate method using 20 mL nutrient agar medium. The molten sterile nutrient agar medium is cooled and mixed thoroughly with 1 mL of culture medium with test organisms and then poured into the sterile petri-dishes and allowed to solidify. On the other hand, filter paper discs (5 mm in diameter) were impregnated with different concentrations of synthesized silver nanoparticles

viz., 20, 40, 60 and 80 μL (1mg/mL) were employed for antimicrobial activity. The antibiotic discs, tetracycline (10 μg) served as positive control for bacteria and fungi respectively. The silver nanoparticles impregnated filter papers were placed on test organism-seeded plates. The distilled water (100 μL) served as control in each plate. Similarly filtered papers impregnated with aqueous plant extracts (30 μL) served as control for comparison. After equilibrium at 4°C, the plates were incubated at 37°C for 24-48 hrs and diameter of any resulting zone of inhibition was measured in milli meter (mm). Triplicates were maintained for all these experiments.

Characterization of silver nanoparticles

X-ray diffraction (XRD) measurement of the plant extract assisted biosynthesized silver nanoparticles were carried out using X'Pert Pro X-ray diffractometer (PAN analytical BV, The Netherlands) equipped with Cu/K α radiation source using Ni as filter at a setting of 30kV/30mA. All X-ray diffraction data were collected under the experimental conditions in the regular angular range. The crystalline size of silver nanoparticle was calculated from the width of the XRD peaks, using a Debye-Scherrer formula: $D = 0.94\lambda / \beta\cos\theta$. The surface morphology of the plant extract reduced silver nanoparticles was examined using Scanning Electron Microscopy (HITACHI SU6000 SEM). Thin films of the samples were prepared on an aluminium foil by dropping a small amount of the sample and placed on a copper grid. Then the samples were characterized in the SEM at an accelerating voltage of 20KV. The plant extract reduced silver nanoparticles powder sample was dried on an aluminium foil coated copper grid and EDX analysis was performed SEM (HITACHI SU6000 SEM) equipped with an EDX attachment.

RESULTS AND DISCUSSION

Novel applications of nanoparticles and nanomaterials are growing rapidly on various fronts due to their completely new or enhanced properties based on size, their distribution and morphology. It is swiftly gaining renovation in a large number of fields such as health care, biomedical, food and feed, drug-gene delivery.^[14]

Green synthesis of silver nanoparticles

The major advantage of plant extracts synthesized silver nanoparticles is that they are easily available, safe and nontoxic in most cases. Hence, the contemporary findings are focused on the green synthesis of silver nanoparticles using the aqueous leaf extracts of *A. radiata*. The colour was changed in the leaf extracts when challenged with 1mM silver

nanoparticles from pale yellow to dark brown within 24 hours (Fig.1). These attained the maximum intensity after 12 hours with increased intensity during the period of incubation indicates the formation of silver nanoparticles.

Preliminary phytochemical analysis

Among the various phytochemical constituents examined, the aqueous leaf extracts of *A. radiata* indicated the presence of flavonoids, cardiac glycosides, tannins, terpenoids, alkaloids and phenols (Table 1)

UV-Vis Spectrophotometer analysis

The absorption spectra of synthesized silver colloids forms a peak centered near 421nm which broadens the peak designates the nanocrystals are polydispersed as per increasing time which is similar to the reports of Nagati et al.^[15] In the present study, the synthesized silver nanoparticles were observed between 200 to 800 nm by using a quartz cuvette with water as reference to evaluate the characterized silver nanoparticles (Fig.2).

Determination of *in vitro* antioxidant activity

Free radical – scavenging ability using DPPH

DPPH radical scavenging activity is a measure of non-enzymatic antioxidant activity. In the present study, all the assessed samples were able to interact intensively with DPPH[•] and reduces the stable violet DPPH radical to the yellow DPPH-H reaching their 50% reductive plateau ranging between 27.2 and 59.1 µg/ mL (Table 2). The reference antioxidant rutin recorded the highest scavenging efficiency towards DPPH radicals (15.8µg/mL), followed by quercetin (16.9µg / mL) and BHA (21.4µg / mL) Table 2 Among the plant extract concentration examined, 4mL concentration of synthesized silver nanoparticle extract possessed effective DPPH radical quenching capacity. Interestingly, these values were significantly lower ($P < 0.05$) than the standard antioxidants tested (Table 2) indicating their superior radical scavenging potential. A fairly correlated findings by Niraimathi et al.^[16] suggests that free radical scavenging ability on DPPH radicals of the silver nanoparticles was found to increase with increase in the concentration.

ABTS radical scavenging activity

In the evaluation of total antioxidant capacity by ABTS^{•+} method, all the sample extracts were able to quench ABTS^{•+} more efficiently with their TEAC values ranging between 169.2 to 430.2 µmol Trolox equivalent/g extract (Table 2). In this context, 4mL concentration of synthesized silver nanoparticle of *A. radiata* leaf extract were found to be

fast and potent scavengers of $ABTS^{*+}$ as they were able to quench $ABTS^{*+}$ more readily than the other solvent extracts. Thus this activity may be endowed by the hydrogen-donating compounds are presumably exist in the polar solvents.^[17]

Determination of antimicrobial activity of silver nanoparticles

Silver nanoparticle has maximum zone of inhibition than the standard disc against *Klebsiella pneumonia* and *penicillium digitalis* whereas, silver nanoparticle has shown same zone of inhibition of Tetracycline against *Bacillus subtilis* and *Serratia marcescens*.(Table 3). Ag NPs can effortlessly influence the nuclear surface of bacteria and they produce a large and remarkable zone because of their size. This may lead to show maximum antibacterial activity. Studies of Bapat et al.^[18] have debated that Ag NPs are liable for their antibacterial activity since silver molecules released from the membrane will eradicate microbes. Similarly, biosynthesised silver nanoparticles may also eradicate fungal spores by destroying their membrane integrity.^[19]

Characterization of silver nanoparticles

X-ray diffraction spectrum of green route synthesized silver nanoparticles revealed that the Braggs reflections were observed in the XRD pattern at $2\theta = 38.92, 44.05, 46.78, 69.01$ and 78.56 . These Braggs reflections clearly indicated the presence of (111), (222), (200), (220) and (311) sets of lattice planes and further on the basis that they can be indexed as face-centered-cubic (FCC) structure of silver (Fig.3). Since, the present study clearly indicated the x-ray diffraction pattern of green route synthesized silver nanoparticles formed crystalline in nature and found around 32nm.

The surface morphology of biosynthesized silver nanoparticles was determined by Scanning Electron Microscope. The SEM image of silver nanoparticles showed spherical in shape with high aggregation of silver particles on the surface of the cell (Fig.4). The silver nanoparticles of uniform distribution have been observed from the SEM image. The result showed that the particles were of spherical shape, with average particle size were 18.18 nm.

The element analysis of the green route synthesized silver nanoparticles was performed using Energy-dispersive X-ray spectroscopy (EDX) spectrum revealed that the green chemistry route obtains silver nanoparticles shown maximum peaks around 3.59 keV correspond to the binding energies of silver ions (Fig.5). Throughout the scanning range of binding energies, some addition peaks belonging to natural compound present in the reaction mixture. There were other EDX spectrum peaks for Cl, K and O suggesting that they are mixed precipitates present in the plant extract and spectrum showed Si element due to preparation of sample for EDX analysis.

Table 1: Qualitative phytochemical analysis of aqueous leaf extract.

Secondary Metabolites	Aqueous leaf
Alkaloids	++
Flavonoids	+++
Terpenoids	++
Cardiac glycosides	+++
Phenols	++
Sterols	-
Saponin	-
Tannin	+++
Resins	-
Reducing sugars	-

Table 2: DPPH and ABTS^{•+} radical scavenging activity of *A. radiata*

Sample	Solvent	Concentration	DPPH IC ₅₀ ($\mu\text{molGAE/mL}$)	ABTS($\mu\text{mol/mL}$)
Leaf	Aqueous	1mL	55.4 \pm 0.1	240.1 \pm 23.1
		2mL	47.1 \pm 0.3	430.2 \pm 24.5
		3mL	34.2 \pm 0.1	308.4 \pm 21.0
		4mL	27.2 \pm 0.4	169.2 \pm 30.2
		5mL	59.1 \pm 0.4	173.0 \pm 12.4

Table 3: Antimicrobial effects of synthesized silver nanoparticles against pathogenic microbes

S.No		Zone of Inhibition (mm)				
		<i>Bacillus subtilis</i>	<i>Serratia marcescens</i>	<i>Klebsiella pneumonia</i>	<i>Salmonella typhi</i>	<i>Penicillium digitalis</i>
1	Tetracycline	12	7	12	10	-
	Control	-	3	-	-	-
2	(Extract) 20 μL	3	4	12	-	3
	40 μL	3	3	10	10	4
	60 μL	3	4	12	-	4
	80 μL	3	4	13	3	5

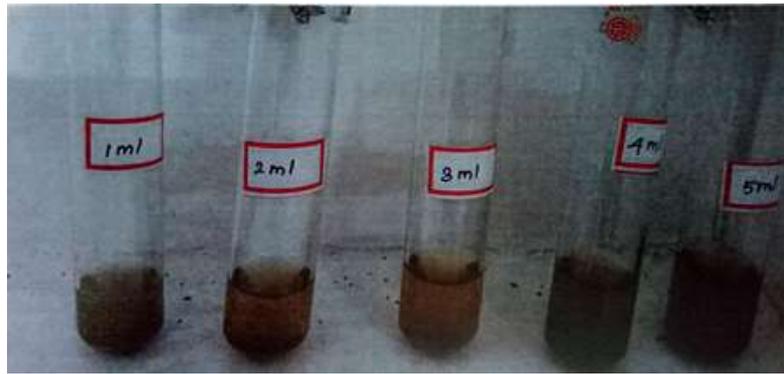
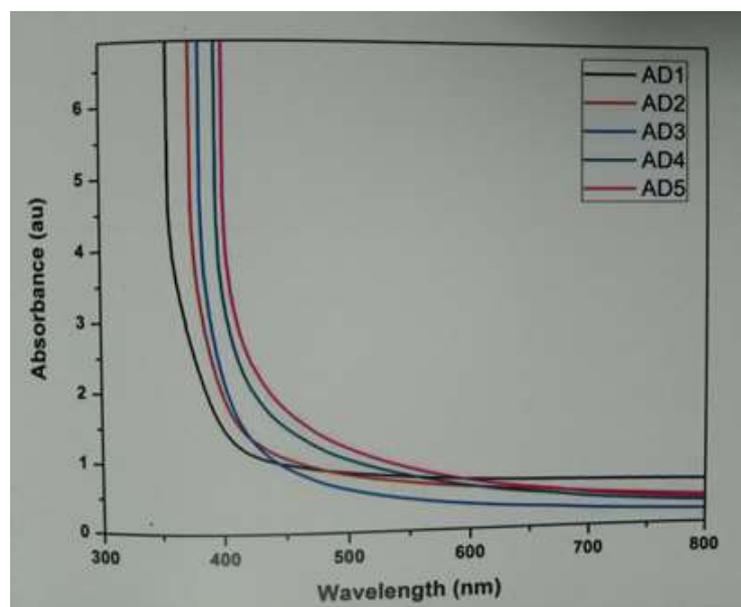


Figure 1: *Actinopteris radiata*, Bedd. leaf mediated green synthesis of AgNPs at various plant extract concentrations



*AD1-1mL Leaf extract, AD2-2mL Leaf extract, AD3-3mL Leaf extract, AD4-4mL Leaf extract, AD5-5mL Leaf extract

Figure 2: UV-Vis absorption spectrum of synthesized AgNPs at various plant concentrations

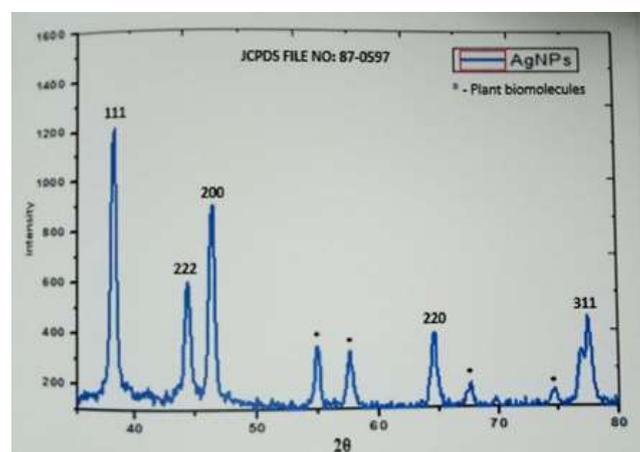


Figure 3: XRD spectrum of synthesized AgNPs at 4mL concentration of the plant extract

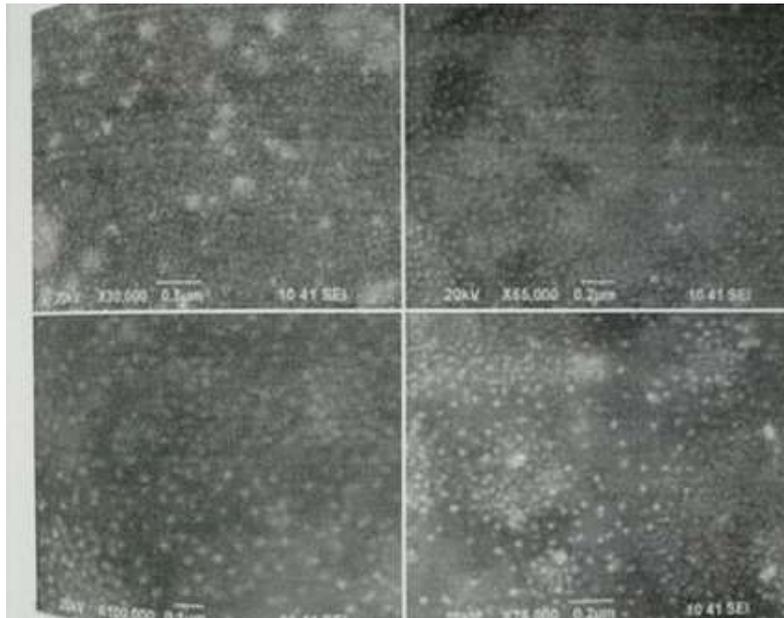


Figure 4: SEM image of biosynthesized AgNPs at different magnification level

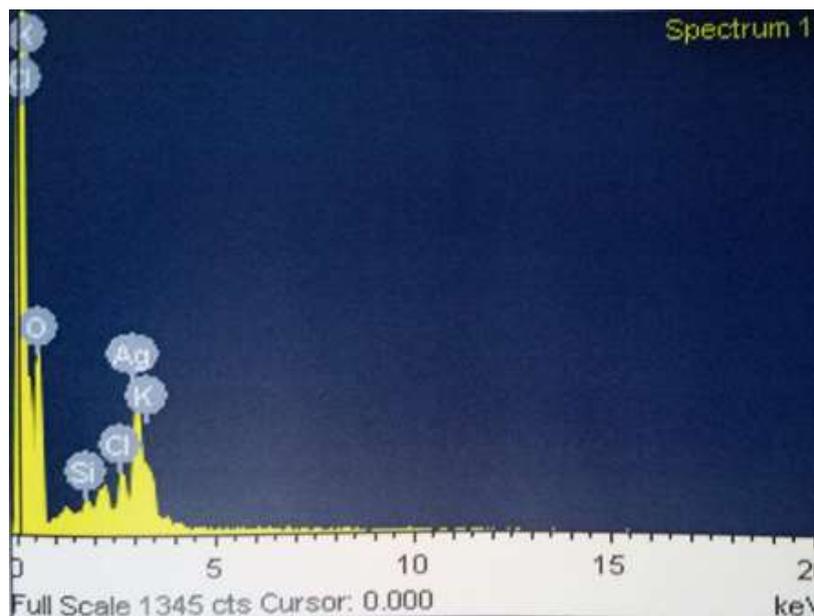


Figure No. 5: EDX spectrum image of biosynthesized AgNPs

CONCLUSION

It is concluded that the aqueous leaf extracts of *A. radiata* displayed a varied amount of biological properties in terms of the assays used for the determination of biological entities. Sufficient number of characterization methods and techniques has been expended for the substantiation of AgNPs. The AgNPs can be biosynthesized as reducing and capping

agent which spectacles varied deviation in shape and size. Moreover, AgNPs have the potential to be widely used in current medical procedures involving nanoparticles fluorescent labelling in immunoassays, targeted delivery of therapeutic drugs and as antibacterial agents in bandages.

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