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Sunlight induced green synthesis of silver nanoparticles using sundried leaves extract of *Kalanchoe pinnata* and evaluation of its photocatalytic potential

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ABSTRACT

In this study, we report a fast and eco-friendly method employing the use of sun dried leaves extract of *Kalanchoe pinnata* (SDKP) for sunlight induced phyto-synthesis of silver nanoparticles. Aqueous extract of SDKP was produced and subjected to phytochemical investigations for determination of alkaloids, phenolics, flavonoids, steroids, glycosides and saponin. Sunlight induced green synthesis of silver nanoparticles was performed by treating the aqueous extract with silver nitrate in different ratios. The reaction was optimized by varying reaction time, temperature. The silver nanoparticles produced were subjected to physicochemical characterization in terms of UV-Vis Spectroscopic studies, Fourier Transform Infra Red spectroscopy (FTIR), Scanning electron microscope, Energy Dispersive Spectroscopy (SEM-EDX), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA), Particle Size Analysis (PS), X-Ray Diffractometer (XRD), X-Ray Diffractometer (XRD), Atomic Absorption Spectroscopy (AAS) and photocatalytic degradation of methylene blue dye. Silver nitrate solution (6 mM) and aqueous extract of SDKP (4% w/v) produced silver nanoparticles of desired properties. The rate of reduction was accelerated within 10 min when the mixture was irradiated with sunlight. This green synthesis was performed at normal pH and room temperature. Silver nanoparticles bear the desired physicochemical properties in terms of optical, structural, thermal and photocatalytic properties. The study provides herein a green approach utilizing sunlight for development of silver nanoparticles using bioactive SKDP plant extract envisages potentiating of therapeutic efficiency of silver nanoparticles. The green approach for development of silver nanoparticles as discussed herein suggests it's applicability for synthesis of other metallic particles.

Keywords: Sunlight irradiation, Silver nanoparticles, *Kalanchoe pinnata*

INTRODUCTION

Currently green synthesis of metallic nanoparticles is an emerging science in the field of nanotechnology and nanomedicine. Due to its wide applicability in various fields, "green chemistry" has been evolved to a widespread scope. Traditionally, chemical reduction has been the most groundwork for synthesis of metallic nanoparticles which often involves use of toxic chemicals are utilized to involve in most of the synthetic protocols [1]. In order to avoid the use of toxic chemicals, organic solvents, several researchers attempt to develop the metallic nanoparticles by environmental friendly and biological approaches. Nanoparticles have been synthesized by biological routes in

green chemistry using bacteria [2], fungus [3], algae [4], enzymes [5], and plant extracts [6] as substitute for conventional chemical synthesis approach.

Kalanchoe pinnata, (family Crassulaceae), is grown as a weed in tropical countries like India, Bangladesh [7]. It is an astringent, sour in taste, sweet in the post digestive effect. It has different Latin synonyms such as *Bryophyllum Calycinum Salisb*, *Bryophyllum pinnatum Linn*. There are diverse common folk names for this plant like miracle plant or air plant, panfuti (Hindi), life plant, love plant, air plant (Mexican), Good luck or resurrection plant, Zakhm-e-hyat, Canterbury bells, Cathedral bells, popularly known as Katakataka for presence of astonishing taste. The leaves of *Kalanchoe pinnata* comprise complex chemical composition including triterpenoids, steroids, polyphenols; flavonoids [7] possess prominent ability to facilitate reduction of silver nitrate required during formation of silver nanoparticles.

Herein, we report for the first time, sunlight irradiated phyto-synthesis of silver nanoparticles using aqueous extract of sundried leaves of *Kalanchoe pinnata*. (SDKP). The developed silver nanoparticles were subjected to different physico-chemical characterization. It was aimed to investigate the role of SDKP extract in the synthesis of silver nanoparticles.

MATERIALS AND METHODS

Aqueous extract of sundried leaves of *Kalanchoe pinnata*:

Fresh leaves of *Kalanchoe pinnata* were dried under sunlight for 15 days and it was ground in an electric mixer to produce fine powder. 2gm of sundried powder of *Kalanchoe pinnata* was dispersed in distilled water and heated at 60°C for 15 min to maximize the extraction of actives. The extract was allowed to cool, filtered and transferred to a suitable container.

Phytochemical screening

Aqueous extract of sundried leaves of *Kalanchoe pinnata* was screened under various phytochemical tests such as alkaloids, phenolics, flavonoids, steroids, glycosides and saponin.

Standard methods described by Evans *et al* [8] were used to test for the presence of phytochemical compound(s) (saponins, tannins, volatile oils, alkaloids, phenols and flavonoids) in the fractions.

Test for alkaloids

0.5 g of extract was dissolved in 5 ml of 1% HCl and kept in a boiling water bath. 1 ml of filtrate was treated with drops of Mayer's reagent. Turbidity is indicated for the presence of alkaloids.

Test for phenolics

0.5 ml of extract was mixed with a few ml of 6% FeCl₃ and appearance of deep green colour confirmed the presence of phenolics.

Test for flavonoids

0.2 ml of extract was dissolved in CH₃OH in test tube individually; heated and a piece of Mg metal added to the mixture, followed by the addition of a few drops of HCl. Appearance of a reddish orange colour is indicator for the presence of flavonoids.

Test for steroids/phytosterols: Liebermann Burchard's test

0.5 ml of extract was dissolved in 3ml of CHCl₃ and filtered. To the filtrate concentrate, H₂SO₄ was added, which formed a lower layer. Reddish brown colour formation is positive result for the presence of a steroid ring.

Test for saponin

0.5 ml of extracts was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for glycosides

Legal's test: Dissolved 0.5 ml of extract in 2ml pyridine and add sodium 2ml nitroprusside solution and make alkaline with sodium hydroxide solution. Pink to red colour solution indicated the presence of glycosides.

Optimization of parameters in green synthesis of silver nanoparticles

Optimization of reaction parameters was performed for better understanding and maximizing the yield of silver nanoparticles with desired results. Concentration of SDKP extract, concentration of silver nitrate, ratio between concentration of SDKP extract and silver salt concentration, reaction time, temperature were considered as different parameters.

Concentration of plant extract

Various concentrations of plant extract (1-6% w/v) were prepared from stock solution by adjusting volume upto 5ml with deionized water in different test tubes. Silver nitrate solution was added in a fixed concentration to these solutions. The test tubes were irradiated to the bright sunlight for 10min and measured spectrophotometrically at 490nm. For that, temperature and reaction time kept constant.

Silver ion concentration

Test tubes containing different concentrations of AgNO_3 (1-9mM) were prepared. To this tube plant extract of concentration 4 % w/v was added. For inducing the phytosynthesis, test tubes were exposed to the bright sunlight for 10min and measured spectrophotometrically at 490nm.

Ratio between concentration of aqueous extract of plant and silver nitrate

Proportion of aqueous extract to silver ion concentration affects the rate of synthesis as well as the size and shape of nanoparticles. Various proportions of silver nitrate solution and plant extract were optimized for desired outcome of silver nanoparticles within nanosize. For inducing the phytosynthesis, test tubes were exposed to the bright sunlight for 10min. Reaction mixture was visually monitored by using different ratio of silver nitrate and leaf extract solution.

Reaction time

To optimize reaction time in view of yield and properties of nanoparticles, reaction mixture in two test tubes containing 4% of plant extract (SDKP) and 6mM AgNO_3 in ratio of 5:2 as per above studied parameters. Reaction mixture was assessed at absorbance of 490nm immediately at 0min, 10 min and after 48hrs. Reaction mixture was exposed under sunlight radiation for 10 min to catalyze of bio-reduction of silver salt.

Temperature

Concentration of silver nitrate was optimized upon earlier parameter as 0.6mM. Reaction test tubes containing plant extract (4%) SDKP mixed with 6mM AgNO_3 were placed in the incubator at temperatures ranging from 25-55°C.

Physicochemical characterization**UV-Vis Spectroscopic studies**

The bio-reduction of Ag^+ ions in solutions was monitored by measuring the UV-VIS spectrum of the reaction medium. The UV-VIS spectral analysis of sun dried leaves of *Kalanchoe pinnata* mediated silver nanoparticles was done by using Shimadzu UV-1800 spectrophotometer at room temperature operated at a resolution of 1 nm with range between 300-700 nm.

Fourier Transform Infra Red spectroscopy (FTIR)

FTIR analysis of the dried silver nanoparticles was carried out through using Nicklet 380 Thermo, US Fourier Transform Infrared Spectrometer.

Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDX)

Each of the colloidal solution containing AgNPs were centrifuged at 4,000 rpm for 15 min and the pellets was discarded and the supernatants were again centrifuged at 5,000 rpm for 30 min. Supernatants were discarded and the final pellets were dissolved in 0.1 ml of deionized water. The pellet was carefully placed on a glass cover slip followed by air-drying. The cover slip itself was screened under scanning electron microscopy (SEM) analysis.

Differential Scanning Calorimetry (DSC)

DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature (Model: Mettler Toledo DSC 822e).

Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA)

TGA-DTA spectra have been recorded in the range from room temperature to higher temperature using simultaneous thermal system. A ceramic (Al_2O_3) crucible was used for heating and measurements were carried out in the suitable condition at the heating rate of $10^\circ\text{C}/\text{min}$.

Particle Size Analysis (PS)

Particle size of phytosynthesized silver nanoparticles was determined by Electrophoretic Light Scattering (ELS) under an applied electric field from the Doppler shift of scattered light for zeta potential determination (Delsa Nano, Beckman Coulter).

X-Ray Diffractometer (XRD)

A glass slide was cut into two half parts by cutter. $50\mu\text{l}$ of phyto-synthesized SDKP silver nanoparticles dispersion was dropped on a glass slide ($3\times 3\text{cm}$) independently and separated on the surface of glass slide to form a thin coat and dried.

Atomic Absorption Spectroscopy (AAS)

Concentration of silver ions was analyzed by atomic absorption spectroscopy (AAS; AA-6300, Shimadzu).

Photocatalytic degradation of methylene blue dye

1mg of methylene blue dye was dissolved to 100ml of double distilled water used as stock solution. About 1mg of phytosynthesized silver nanoparticles was added to 10ml of methylene blue dye solution. A control was prepared in similar manner without addition of silver nanoparticles. Before exposing to irradiation with sunlight, the reaction suspension was well mixed. Afterwards, these dispersions were put under the sunlight and monitored in interval of time period of 30min . At specific time intervals; these suspensions were measured against absorbance at 660nm to evaluate the photocatalytic degradation of dye.

Percentage of dye degradation was estimated by the following formula:

% Decolourization = $(\text{Co}-\text{C})/\text{Co} \times 100$, where Co is the initial concentration of dye solution and C is the concentration of dye solution after photocatalytic degradation.

RESULTS**Preliminary phytochemical screening**

Aqueous extract of *K. pinnata* were confirmed for the presence of secondary metabolites / phytochemicals under preliminary phytochemical tests in Table 1. Most of phytochemicals play an important role as capping agents which stabilizes the silver nanoparticles in order to prevent in agglomeration and increasing particle size.

Optimization of parameters

After optimization / fixation of factors relating with reaction of silver nitrate with the aqueous extract of SDKP, 6mM silver nitrate solution was selected and aqueous extract of SDKP leaves chosen to initiate the phytosynthesis of silver nanoparticles about 4% respectively in the ratio of $5:2$ v/v. Absorbance of silver nanoparticle colloidal solutions has shown a high difference between absorbance 0.132 at 0min and 0.456 at 10min . It is confirmed that sunlight irradiation provides a swift velocity of bio-reduction within a short period of time. Agglomeration of silver nanoparticles after 48hrs of reaction was observed indicating as a sign of instability. At 45°C , absorbance of silver nanoparticles colloidal solutions have shown 0.285 as its lower value and thereafter its absorbance begins decline with increasing temperature. Fig.1 and Fig.2 depict the preparation of different concentration of this plant extract. Table 2 and Fig.3 illustrated the optimization of various parameters.

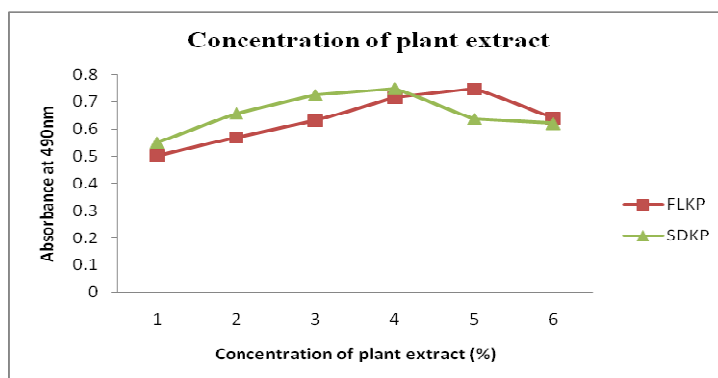


Fig.1: Concentration of plant extract against absorbance at 490nm

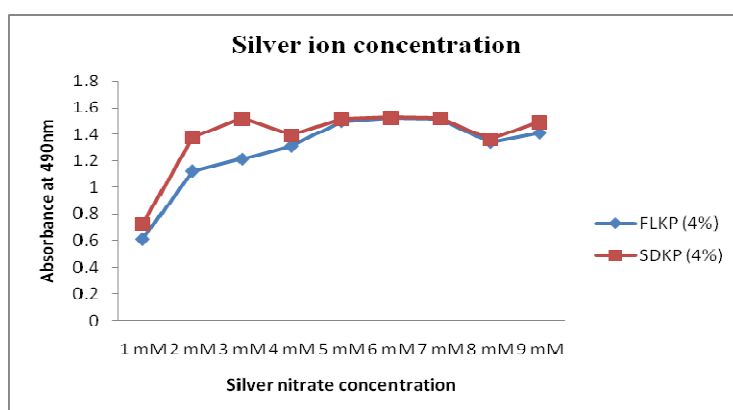


Fig. 2.: Concentration of silver ion solution against absorbance at 490nm



Fig. 3.: Optimization pattern during exposure to sunlight

Physicochemical characterization

UV-Vis Spectroscopic studies

UV spectroscopy is a suitable, preliminary method for characterization of Ag nanoparticles based on optical properties called Surface Plasmon Resonance (SPR) [9]. Addition of sundried leaves of *Kalanchoe pinnata* aqueous

extract resulted in the colour change from transparent to reddish brown. The colour changes arise from the excitation of SPR with the silver nanoparticles. The SPR of silver nanoparticles produced a peak centered near to 420 nm. During green synthesizing, the process of reaction was monitored by UV. Bio-reduction reaction of silver nitrate solution to silver by interacting with leaf extract has been screened by the UV-Vis spectroscopy ranging from 300 to 700 nm in the present study. The maximum absorption was observed nearby at 421 nm indicating the successful conversion of silver nitrate (Ag^+) to silver nanoparticles (Ag^0) shown in Fig. 4.



Fig. 4.: Silver nanoparticles solution shown SPR at absorbance at 450nm

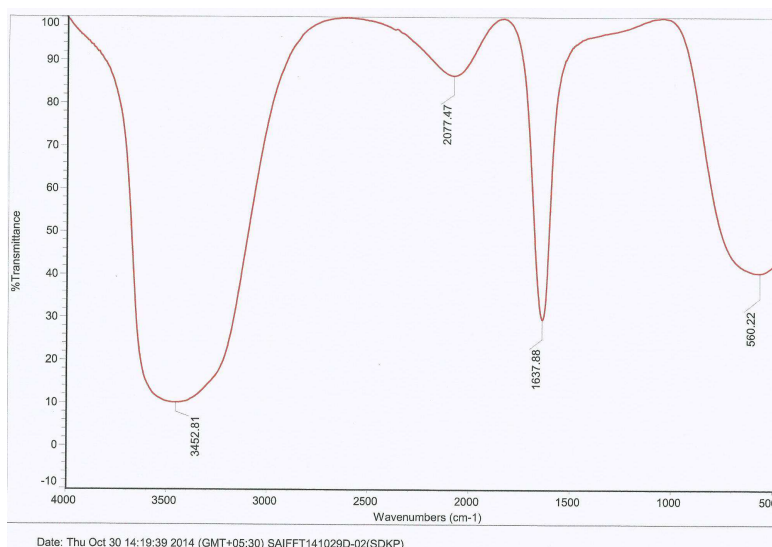


Fig. 5: FTIR image of SDKP silver nanoparticles

Fourier Transform Infra Red spectroscopy (FTIR)

In the present study, FTIR spectra were used to identify the potential biomolecules present in the extract which is responsible for reducing and capping the phytosynthesized silver nanoparticles. An FTIR spectrum is useful in probing the chemical composition of the surface of the silver nanoparticles [10]. In Fig. 5, FTIR spectrum of phytosynthesized silver nanoparticles showed the two strong IR bands of hydroxyl and phenols (3453.06 cm^{-1}), whereas C=C stretch of benzene and amide-I linkage (1637.76 cm^{-1}) and other IR bands of (2076.95 cm^{-1}) and (559.46 cm^{-1}). The broad band appearing at (3453.06 cm^{-1} and 3452.81 cm^{-1}) are assigned for O-H stretching vibration indicating the presence of hydroxyl and phenolic hydroxyl groups, responsible as the reducing as well as capping agents. Absorption peak (1631.39 cm^{-1} and 1631.27 cm^{-1}) in the infrared region of the electromagnetic spectrum exhibits the binding of amide linkage with silver nanoparticles which may be assigned to the carbonyl

stretch in proteins and clearly indicates the presence of protein as capping agent for silver nanoparticles. Proteins have stronger affinity to bind silver nanoparticles which increases the stability of synthesized nanoparticles [11]. These results confirmed that carbonyl group of amino acid residues has strong binding ability with metal leading to the formation of layer adsorbed on the surface metal nanoparticles as capping agent to prevent agglomeration [12].

Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDX)

Phytosynthesized silver nanoparticles as polycrystalline structure were revealed as shown in Fig.6 and Fig.7. Structural analysis by SEM together with EDX showed 84.85nm in size of silver nanoparticles.

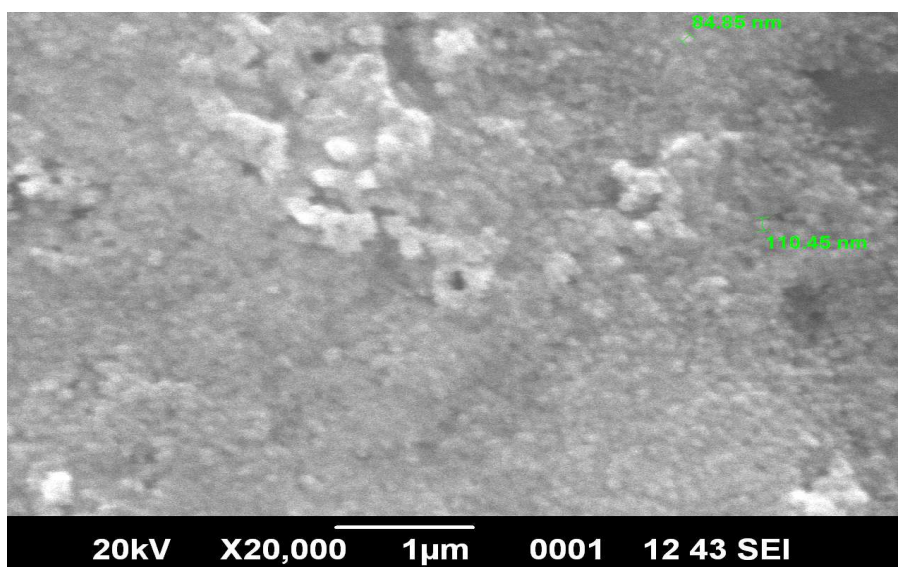


Fig. 6: SEM image of SDKP silver nanoparticles

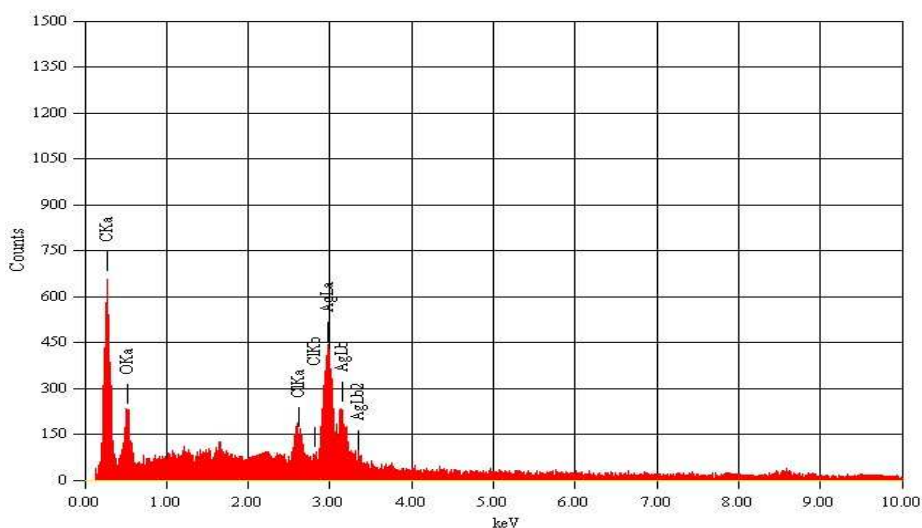


Fig. 7: EDX spectra of SDKP silver nanoparticles

Differential Scanning Calorimetry (DSC)

DSC study displays the nature of the plant extract compounds loaded on the nanoparticles. Different compounds show their particular characteristic endothermic peaks in DSC [9],[13]. The well pointed DSC curves of AgNPs of SDKP are shown in Fig.8. AgNPs of SDKP due to transition temperature (T_g). The denaturation enthalpy of both of AgNPs is closer to single stage decomposition. Earliest weight loss for AgNPs took place at above 100°C. Therefore, the denaturation temperature displayed in the DSC curves for AgNPs suggests that the phytochemicals

present in the extract which are responsible for the reduction of Ag^+ to Ag^0 (nanoparticle) could be a highly thermal stable compound.

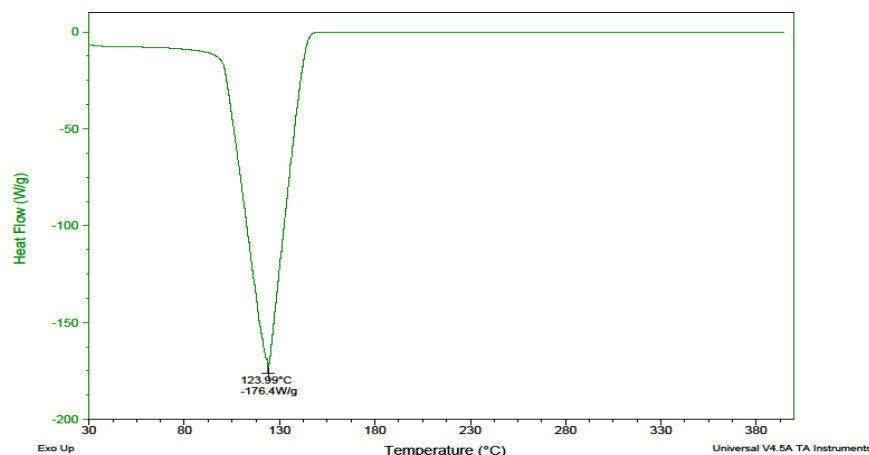


Fig. 8: DSC spectra of SDKP silver nanoparticles

Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA)

TGA and DTA curves of silver nanoparticles of sun dried leaves of *Kalanchoe pinnata* extract are shown in Fig.9. It is observed from TGA-DTA curve of SDKP shown at below 60°C. Loss of water and organic components are responsible for this. DTA provide the complete thermal decomposition and crystallization profiles of the silver nanoparticles [13].

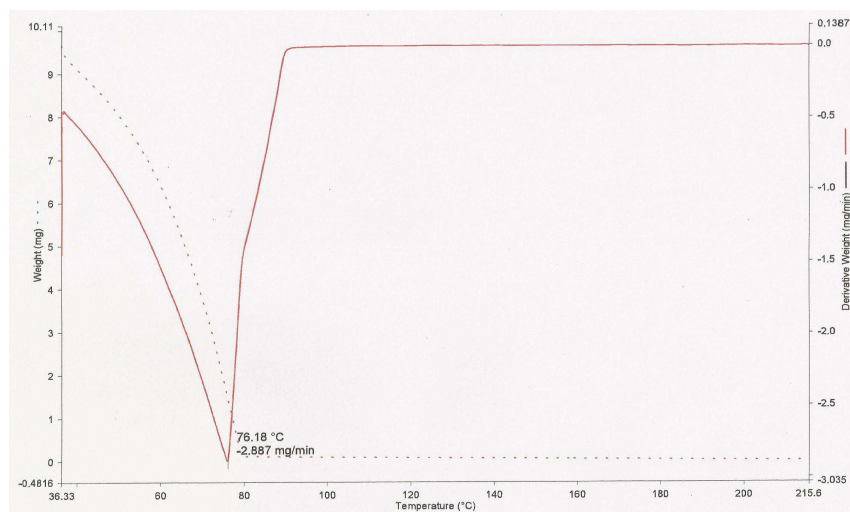


Fig.9: TGA-DTA spectra of SDKP silver nanoparticles

Particle Size Analysis (PS)

The particle size determination of phytosynthesized silver nanoparticles was shown based on intensity. Particle size analysis of sun dried leaves of *Kalanchoe pinnata* extract mediated silver nanoparticles has shown in Fig.10 respectively.

The mean particle size (z-average), polydispersity index (PI) of phytosynthesized silver nanoparticles was determined.

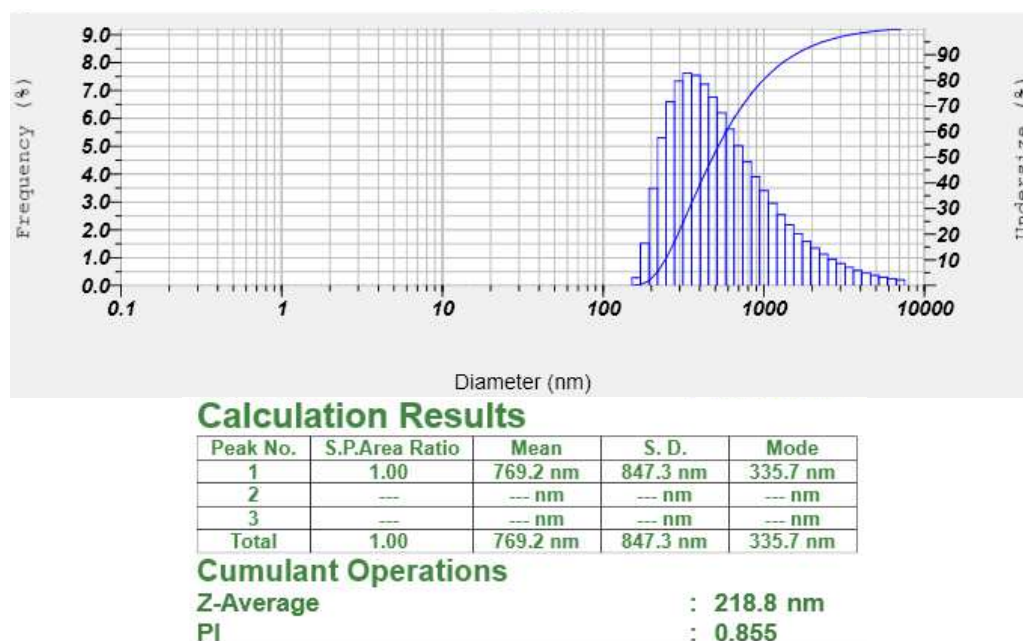


Fig. 10: Particle size analysis of SDKP silver nanoparticles

X-Ray Diffractometer (XRD)

X-ray diffraction patterns were recorded in the scanning mode on an X'pert PRO PAN analytical instrument operated at 40 KV and a current of 30 mA with Cu K α radiation ($\lambda=1.5406 \text{ \AA}$). Different diffraction intensities were recorded from 35° to 50° , in 2θ angles. The diffraction intensities were compared with the standard JCPDS files. The software gave the information about the crystal structure of the particle.

The average size of the nanoparticles can be estimated using the Debye–Scherrer equation [14]:

$$D = k \lambda / \beta \cos \theta,$$

Where, D = Thickness of the nanocrystal, k = constant, λ = wavelength of X-rays, β = width at half maxima of (111) reflection at Bragg's angle 2θ , θ = Bragg angle.

XRD pattern thus clearly illustrated that phytosynthesized silver nanoparticles are confirmed for crystalline in nature as shown in Fig. 11.

Atomic Absorption Spectroscopy

Silver nanoparticles suspensions were analyzed by atomic absorption spectroscopy, which showed that the negative result of unreduced silver ions indicating the completeness in bio-reduction of silver nitrate (Ag^+) to silver nanoparticles (Ag^0). It is confirmed that silver nanoparticle has zero valence in shell hence it has shown negative value in the result. Our result is in concurrence with the reported article [15].

Photocatalytic degradation of methylene blue dye by silver nanoparticles

Dye degradation was observed initially by colour change. At starting point the colour of dye shows deep blue colour, changed into light blue after the 1 hour of incubation with silver nanoparticles while exposed to sunlight shown in Fig. 12. Thereafter light blue was changed into light green. Finally, the degradation process was completed up to 72 hours and was identified by the change of reaction mixture colour to colourless. Photocatalytic effect of silver nanoparticles on degradation of dye was demonstrated with the help of methylene blue dye. The degradation study of methylene blue was carried out by use of silver nanoparticles in the different time interval period in exposure of sunlight. Absorption spectrum for methylene blue dye was decreased gradually with the increase of the exposure to sunlight designating the photocatalytic degradation reaction of methylene blue. The percentage of

degradation efficiency of sun dried leaves of *Kalanchoe pinnata* extract mediated silver nanoparticles were calculated as 41.44% and 41.63% respectively after 72 hours shown in Fig. 13.

SDKP

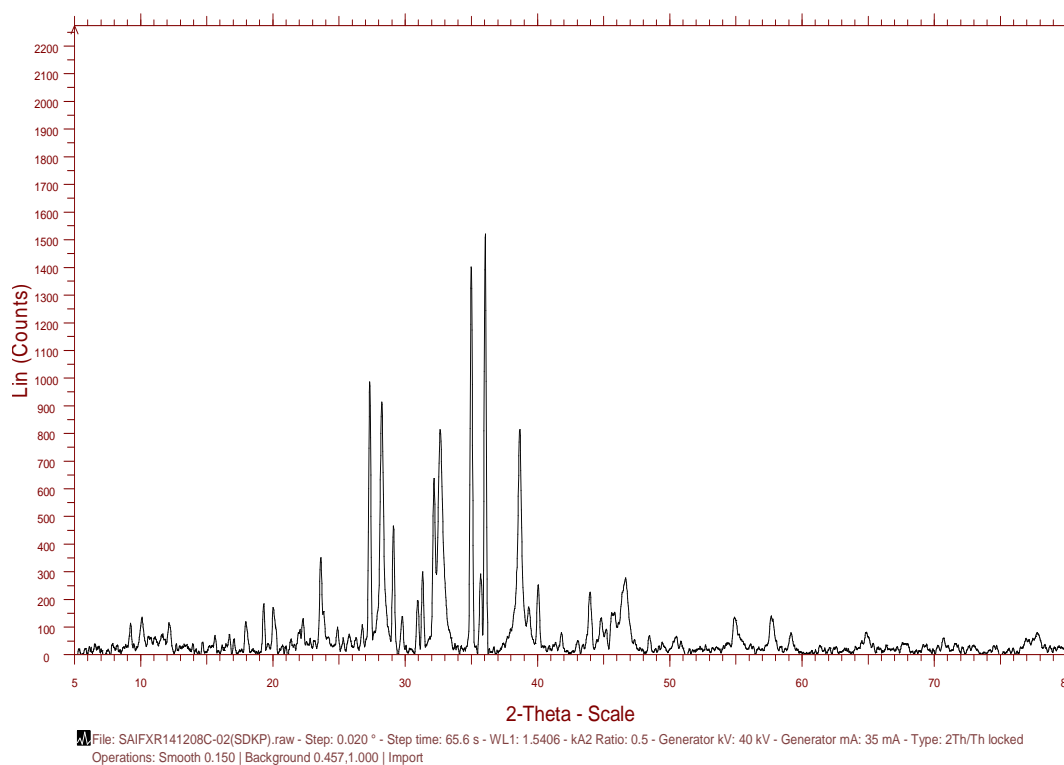


Fig. 11: XRD spectra of SDKP silver nanoparticles



(Fig. 12: Photocatalytic degradation studies of SDKP silver nanoparticles Green colour indicates the presence of silver nanoparticles in methylene blue solution; Blue colour indicates the absence of silver nanoparticles methylene blue)

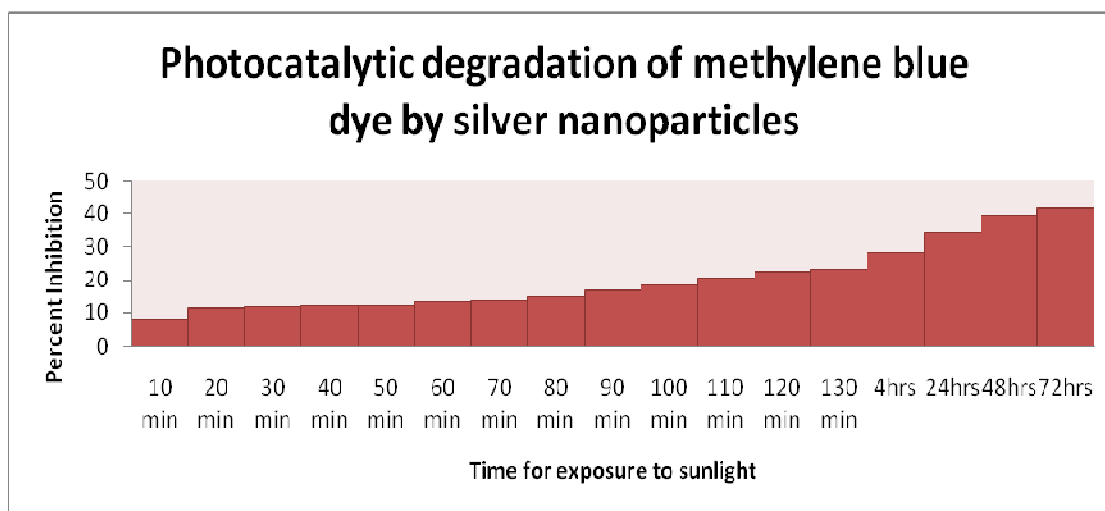


Fig. 12: Photocatalytic degradation studies of SDKP silver nanoparticles

DISCUSSION

Our preliminary pilot study has been revealed that by this plant extract takes much longer incubation about 2 hours for synthesis of silver nanoparticles under dark condition. In contrast, we have been observed that in presence of sunlight, it takes little incubation time to reduce silver nanoparticles about 10 minutes which minimized reaction time in a great deal. So we have taken this objective to synthesize silver nanoparticles based on the strategy of sunlight irradiation.

The mechanism of sunlight irradiation strategy has been explained that the solar photons hit the nanoparticles present in the reaction mixture during exposure in sunlight, the electrons at the particle surface are excited and reacts with dissolved oxygen molecules in the reacting medium and converted into oxygen anion radicals. These radicals break the organic dye into simpler organic molecules leading to the rapid degradation of the dye [16]. Therefore, the phyto-synthesized silver nanoparticles may act as a stable and efficient photocatalyst for degradation of methylene blue under visible light irradiation supported with reported paper [16].

It is reported first in the present study to combine use of sun dried leaves of this plant in aid of sunlight irradiation to initiate green synthesis of silver nanoparticle. Sun dried leaves provide desired outcomes and benefits of sunlight irradiation has been well documented in according to some few cited literatures, sun dried leaves of plant extract provide the smallest size of silver nanoparticles rather than fresh leaves extract which has reported that some authors have worked on the use of sun dried leaves of plant for green synthesis of silver nanoparticles recently [17]. Advantages for use of sun-dried leaves of the plant and its simplicity and suitability have been explained by researchers [18], [19], [20], [21], [23] and benefits of sunlight irradiation strategy [20]. In our study, pH parameter has not incorporated to study in the optimization as the synthesis of silver nanoparticles was carried out in neutral pH with respect to the reported paper [23]. Phytosynthesized silver nanoparticles are confirmed to have a good catalytic activity on the reduction of methylene blue due to decrease in absorbance value with respect to time. Electron relay effect of silver nanoparticles is characteristic for degradation of dye. This result is agreement with reported paper [24].

Advantages of the study:

This study may be providing many advantages in view of environment friendly approach:

1. Green synthesis may be helpful to be pollution reducing agent, eco-friendly by avoiding the use of hazardous chemicals to prepare silver nanoparticles
2. Application of solar irradiation strategy acts as a catalyzing agent in the photochemical reaction of extract and silver salt solution which is cheap, cost free.
3. Photocatalytic studies of methylene blue by phytosynthesized silver nanoparticles will be envisaged to degrade in the organic dye in the textile, to detoxify organic toxins in wastewaters through photocatalytic reaction.

4. Synthesis of phyto-synthesized silver nanoparticles at inferior cost with natural energy will promote functionalized fabrication on industrial stage and applicable for medical textiles manufacturing [25].

Despite of that aqueous extract prepared from fresh leaves or sundried leaves are varied in the phytoconstituents, phenolic, flavonoid, saponin contents and other active biological compounds may vary bio-reduction interaction. Many contributing factors like temperature, geographical location, and change in climate affect on the growth of plant and effect of drying on plant used for bio-reduction which is difficult to synthesize silver nanoparticles under identical conditions.

It should be investigated in further studies of utilizing fresh leaves of this plant to synthesize silver nanoparticles to find which of the extract either of sun dried or fresh leaves will be most suitable for green synthesis of silver nanoparticles.

CONCLUSION

In the current study sunlight induced phytosynthesis of silver nanoparticles was successfully achieved using SDKP extract. Silver nanoparticles bear the desired physicochemical properties in terms of optical, structural, thermal and photocatalytic properties. The study provides herein a green approach utilizing sunlight for development of silver nanoparticles using bioactive SKDP plant extract envisaging potentiation of therapeutic efficiency of silver nanoparticles. The green approach for development of silver nanoparticles as discussed herein suggests it's applicability for synthesis of other metallic particles.

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