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In-Vitro Bio-Fabrication of Silver Nanoparticle Using *Trigonella foenum* Seed Extract.

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ABSTRACT

Rapid synthesis of silver nanoparticles through economically feasible green chemistry approach is highly desirable. It is well known that on treating the metallic salt solution with some plant extracts, a rapid reduction occur leading to the formation of highly stable metal nanoparticles. In this study we have developed a novel method to synthesize silver nanoparticles by mixing silver salt solution with seed extract of *Trigonella foenum* (Methi) without using any surfactant or external energy. In this method, physiologically stable, bio-compatible AgNPs were formed. These functionalized AgNPs could be used for targeted drug delivery with enhanced therapeutic efficacy and minimal side effects. With this method rapid synthesis of nanoparticles was observed to occur; i. e. reaction time was 1 to 2 hours as compared to 2 - 4 days required by microorganisms. These nanoparticles were analyzed by various characterization techniques to reveal their morphology, and chemical composition. TEM image of these NPs indicated the formation of spherical, non-uniform, poly-dispersed nanoparticles.

Keywords: Green synthesis, AgNPs, XRD, SEM, *T. foenums*.

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INTRODUCTION

It is known that animate and inanimate body consist of atoms. An atom is so small that it is beyond our perception. However at the end of the 20th century, with the breakthrough of nanotechnologies, we realize that that the nanometric size, close to that of atoms, is no longer beyond our perception. Nanomaterials have a long list of applicability in improving human life and its environment. It has been found that a 5000 years old Indian system of medicine *Ayurveda* had some knowledge of nanoscale fabrication. Thus we can claim that nanotechnology was in existence even before this was coined.

A bulk material has constant physical properties regardless of its size, but at the nanoscale often this is not true. Several well characterized bulk materials have been found to alter their properties when studied at the nanoscale. There are many reasons for this including the fact that nanoparticles possess a very high aspect ratio i.e. surface to volume ratio. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic and molecular structure. Metallic nanoparticles are intensely studied due to their unique optical, electrical and catalytic properties. To utilize and optimize chemical and physical properties of nano sized metal particles, a large spectrum of research have been focused to control the size and shape which is crucial in tuning their properties. Several approaches are in practice to synthesize the metallic nanoparticles such as UV irradiation, laser ablation, aerosol technology, lithography, ultrasonic fields, and photochemical reduction techniques. However these techniques are expensive and may involve the use of hazardous chemicals. To switchover these technical hitches biological principles has been established. It was demonstrated that parallel to the chemical process, it is also possible to synthesize gold nanoparticles of uniform size extra-cellularly using *Fusarium oxysporum* and *Aloe vera*.

The technique for obtaining nanoparticles using naturally occurring reagents such as vitamins, sugars, plant extracts, and biodegradable polymers could be considered attractive for nanotechnology. Silver nanoparticles can be produced either intra-or extra- cellularly by using living organism. Recently Shahverdi A.R. *et al.* have synthesized the noble metallic nanoparticles by gram negative bacterium *Klebsiella pneumoniae*. Also fungi such as *Verticillium*, *Fumigatus*, *Trichoderma*, *Asperillum*, *Phapnerochaete Chryso sporium* have been explored for noble metallic nanoparticles synthesis. Numerous reports have been published in literature related to various aspects of bio-mineralization.

Recently synthesis of metal nanoparticles using plants is being looked into with interest. While micro-organisms continue to be investigated for metal nanoparticles synthesis, the use of plant extracts is an exciting possibility and is relatively unexplored and under-exploited. Among the various biological methods used for nanoparticles synthesis plant based materials seem to be best candidates and they are suitable for large scale biosynthesis of nanoparticles. Plant parts such as leaf, roots, latex, seed and stem are being used for metal nanoparticles synthesis. Plants have been known to bio-mineralize calcium carbonate, silica and even magnetite internally. Certain plants are known to hyper-accumulate these heavy metals or can even be induced to hyper-accumulate within different parts of plants. The internal accumulation of metal in plants can occur both via complexation of the metal ion with a suitable bio-ligand in its native oxidation state or after

its reduction to a lower oxidation state. The possibility of reduction of metal ions by plants and the presence of metal complexing agents in them entices a materials scientist to use plants for the goal of synthesizing nanoparticles and controlling their size and shape and to experiment on the likelihood of forming nanoparticles of low reduction potential metals. The possibility of synthesizing nanoparticles of different compositions using plants would offer an environmentally friendly alternative to the existing potentially toxic chemical and physical methods of preparations. This would also help stave-off the growing apprehensions related to environmental degradation and biological hazards apart from being a cost effective process with potential to scale up for large scale synthesis. In fact Jose-Yacaman and co-workers have shown that live alfalfa plants when supplied with Au^{3+} ions reduce them to Au^0 state and absorb them resulting in the internal formation of gold nanoparticles. Mallikarjuna K. *et al.* have synthesized silver nanoparticles using leaf broth of *Ocimum santum* as reducing and stabilizing agent. While these reports demonstrate the possibility of synthesizing metal nanoparticles using plants, they suffer with the inherent complication of being intracellular, making the isolation of particles an additional difficult job. To overcome the difficulty in intracellular approach, an extracellular synthesis is desired. Here, extract from well known commercial products like *Coffea Arabica*, and *Cymbopogon citrus* were used as green reagents in AgNPs synthesis. Thus by reducing silver nitrate in solution of tea extract or epicatechin of varying concentrations, spherical silver nanoparticles were formed that had controllable size distribution depending upon the concentration of tea extract and epicatechin in the samples. Solution temperature, concentration of metal salt and reducing agent and reaction time etc influence the shape and size of particle. Amongst metal nanoparticles.

The green chemistry route is more advantageous because it does not require elaborate process such as multiple purification steps, maintenance of microbial cell culture etc. In green chemistry approach of synthesis the reaction is normally carried out at room temperature although in some processes a little heating below 100°C was applied. High amount of phenolic compounds in the plant extract are generally supposed to influence the reduction process and stabilize nanoparticles preventing agglomeration. It is well known that few medicinal plants exhibit anti-oxidant property. Thus they can act as biological source of reducing agent. On this belief choice of plants for this purpose were those carrying medicinal and aromatic properties. S. Shiv Shankar has reported synthesis of silver and gold nanoparticles using decoction solution of *Azadirachata indica*.

In the present research work *Trigonella foenum* seed extract have been used as a reducing agent. The same extract also acts as a capping agent. *T. foenum* is widely available plant in tropical country like India. The aqueous extract contains protein which may act as a bio-ligand. This method gives nanoparticles well separated from each other and no aggregation was observed. Similarly the anti-microbial activity was also studied. A thorough study on literature of *T. foenum* reveals that the major components of the plant are protein in which globulin and histidine are major components and also contains albumin and Phosphorus. The seed contains Trigonelline ($\text{C}_7\text{H}_7\text{O}_2\text{N}$), Choline, fatty acids, phosphates, lecithin, and nucleo-albumin. Therefore it is as nutritive as cod-liver oil. It is also used as a *Ayurveda* medicine against indigestion, bleeding piles, galactagogue, diarrhoea, griping pain, anaemia, diabetes, goitre, leucorrhoea, and as appetizer and purgative and in treating eye diseases [6, 16].

Silver Nanoparticles

AgNPs exhibit tremendous applications in various areas such as biomedical field, spectral selective coatings for solar energy absorption, optical receptors, bio-labeling, intercalation materials for electrical batteries, filters, antimicrobial agents, textile industries, water treatment, sunscreen lotions and sensors etc [1-7]. They are widely used as a catalyst for the oxidation of methanol to formaldehyde and carbon monoxide to carbon dioxide and are useful substrate for Surface Enhanced Raman Spectroscopy (SERS). Chemical reduction is most frequently applied method for the preparation of AgNPs. Commonly used reductants are borohydride, hydrazine hydrate, ammonia and elemental hydrogen. The reduction of silver ions in aqueous solution generally yields colloidal silver with particle diameter of several nano-meters. Initially the reduction of Ag^+ ions leads to the formation of silver atoms (Ag) which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal Ag particle. When the colloidal particles are much smaller than the wavelength of visible light, the solution have a yellow colour with an intense band in the 380-430nm range and other less intense or smaller bands at longer wavelength in the UV-visible absorption spectrum.

Previous studies have revealed that use of strong reducing agents such as boro-hydride, hydrazine hydrate resulted in small particles that were somewhat mono-disperse and use of weaker reducing agent such citrate resulted in slower reduction rate with larger particle size. Thus the correct solution is the use of moderately strong reducing agent and plant extract fits in this category.

MATERIAL AND METHOD

AgNPs were prepared by using *T. foenum* seed extract. *T. foenum* seeds have been purchased from local market Jaysingpur, India. 50gm of seeds have been taken for preparation of extract. The obtained seeds were sieved and impurities were removed. It is thoroughly washed in running water and finally rinsed with deionized water until no foreign material remained behind. After washing, the collected seeds were kept in 100ml of deionized water over night and macerated. Then it was finely crushed in mortar and filtered by using cotton and collected in stoppered glass bottle. The extract is diluted to 250ml using deionized water and the solution was kept in refrigerator at 4°C temperature and later used as a reducing agent as well as capping agent. The AgNO_3 used in reaction was analytical grade chemical obtained from Sigma Aldrich and used without further purification. Redistilled deionized water was used for sample preparation.

Then, 100ml of 0.1N AgNO_3 solution was taken in 500ml beaker. Then 50ml of *T. foenum* seed extract solution was taken in burette and drop wise added at room temperature with constant stirring. As soon as the extract solution was added dark precipitate appeared in the solution. After complete addition of 50ml of solution a sufficient amount of the precipitate was observed and it was separated by high speed centrifugation at about 8000rpm. The separated solid mass was washed with alcohol for few times to remove organic alcohol soluble impurities. After complete washing the solid mass was kept

in oven for drying. The complete drying of this solid mass resulted in a black coloured material which was powdered in mortar and sampled for characterization purpose.

RESULT AND DISCUSSION [8-18]

Biosynthesis of silver nanoparticles was indicated based on colour developed in solution during the course of reaction. The intensity of colour interprets the degree of bio-reduction of AgNPs due to addition of *T. foenum* seed extract. Formation of AgNPs was primarily recorded based on surface plasmon resonance peak in UV-visible spectroscopy.

UV Spectroscopic Analysis

The blackish brown coloured sample powder was dissolved in deionized water and sonicated. Then this solution was taken in cuvette and exposed to UV-visible radiation and the absorbance of the solution was recorded. Because of the surface plasmon resonance phenomena resonant peak occurs at different wavelength for different nanoparticles solution and as per the theory of resonance maximum wavelength is absorbed at resonant wavelength. It is reported in the literature that typical AgNPs shows the characteristic SPR at the wavelength in the range of 380-440nm. Here from fig. 1 we observe SPR for the sample solution to occur at the wavelength of 421 nm which confirms the presence of Silver nanoparticles in the prepared solution. The SPR absorbance is sensitive to the nature, size and shape of particles present in the solution and also it depends upon their inner particle distance and the surrounding media.

The MATLAB analysis of this UV image shows that the image format is JPEG with bit-depth of 24 bits and image size is 492 x 492 with 13.7 KB. Also it is seen that the image class is uint8. This UV analysis is done at Department of Chemistry, Shivaji University, Kolhapur.

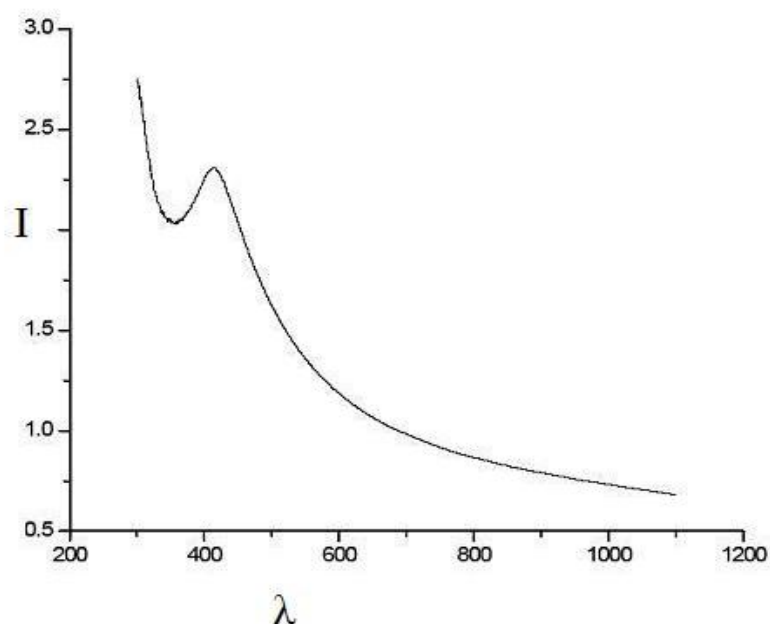


Figure 1: UV Spectroscopic graph showing absorbance of silver nanoparticles

XRD Pattern

XRD patterns of synthesized AgNPs are shown in fig. no. 2 where four major peaks appeared. The peak position explains about the translational symmetry namely size and shape of the unit cell whereas the peak intensities give details about the electron density inside the unit cell. These peaks correspond to cubic structure which is in agreement with the JCPDS file No. 00-004-0783 and this indicates that the synthesized nanoparticles are crystalline in nature.

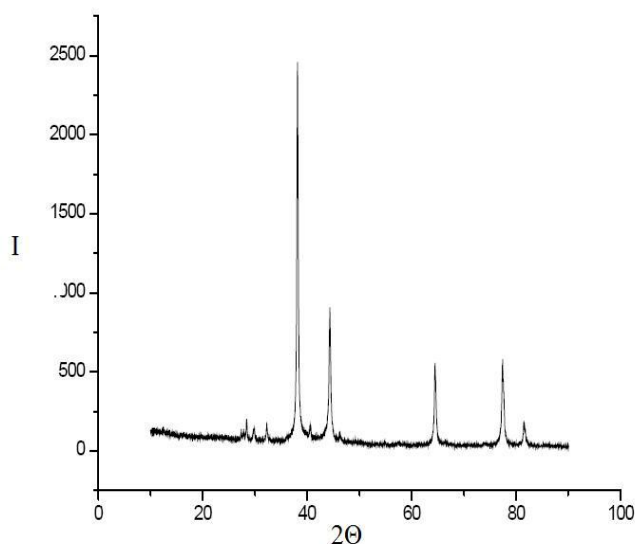


Figure 2: XRD pattern of synthesized nanoparticles

Particle Size Distribution Analysis

Particle size distribution histogram shown in fig. 3 indicates that 10.07% particles size is around 13nm with a standard deviation of 16.48% size while the remaining particles are around 633.5nm with a standard deviation of 20.58%. The difference in size as observed from TEM and PDS may be due to presence of bio active molecules of *T. foenum* on AgNPs surface. Prathna *et al.* found the particle size of AgNPs in the following order given by name of technique: DLS> AFM>TEM>XRD and our observations are concurrent with this. The MATLAB analysis of PSD image shows the bit-depth of 24 bits and image resolution of 1024 x 768. Also the image file-size was 124.9KB and image format JPEG with image class as uint8. The PSD analysis is done at Dr D. Y. Patil University, Kolhapur, India.

SEM Analysis

The SEM image given in fig. 4 reveals the formation of cluster of spherical beadlike structure of AgNPs with non uniform distribution. The MATLAB analysis gives the pixel depth of the image equal to 8 bits and the image resolution of 1280 x 960. Also the image file-size was found to be 1.23MB and the image format as TIFF. The SEM image has been taken with JSM-6360 instrument which uses accelerating voltage of 20KV. The SEM imaging has been done at Department of Physics, Shivaji University, Kolhapur, India.

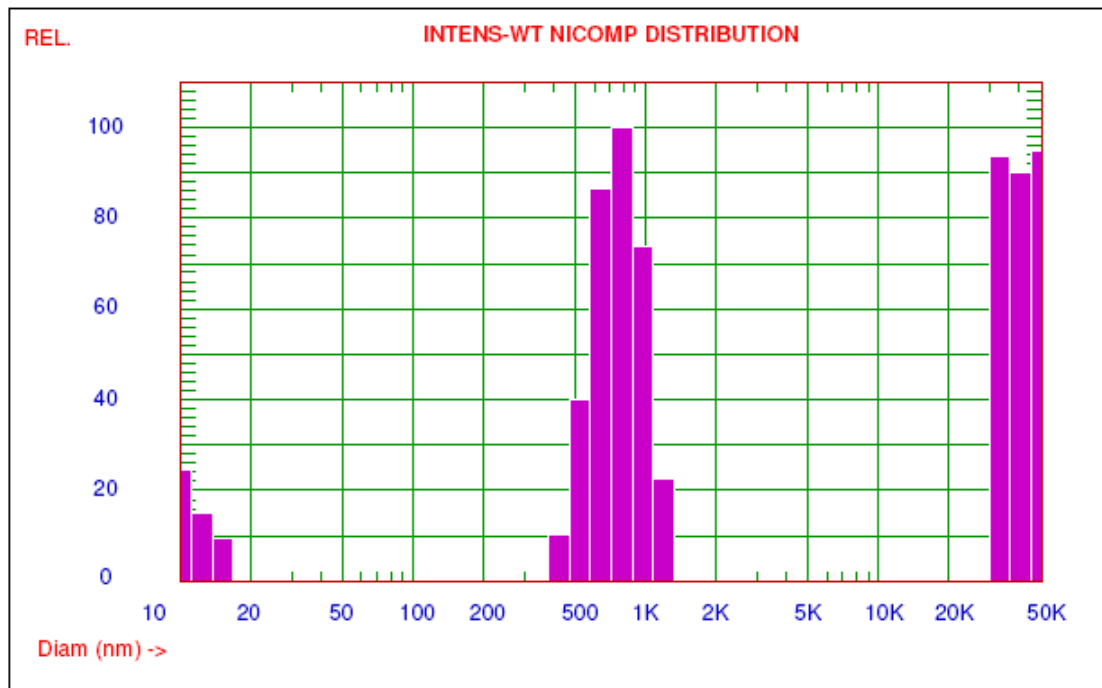


Figure 1: Graph showing Particle size distribution for Ag nanoparticles

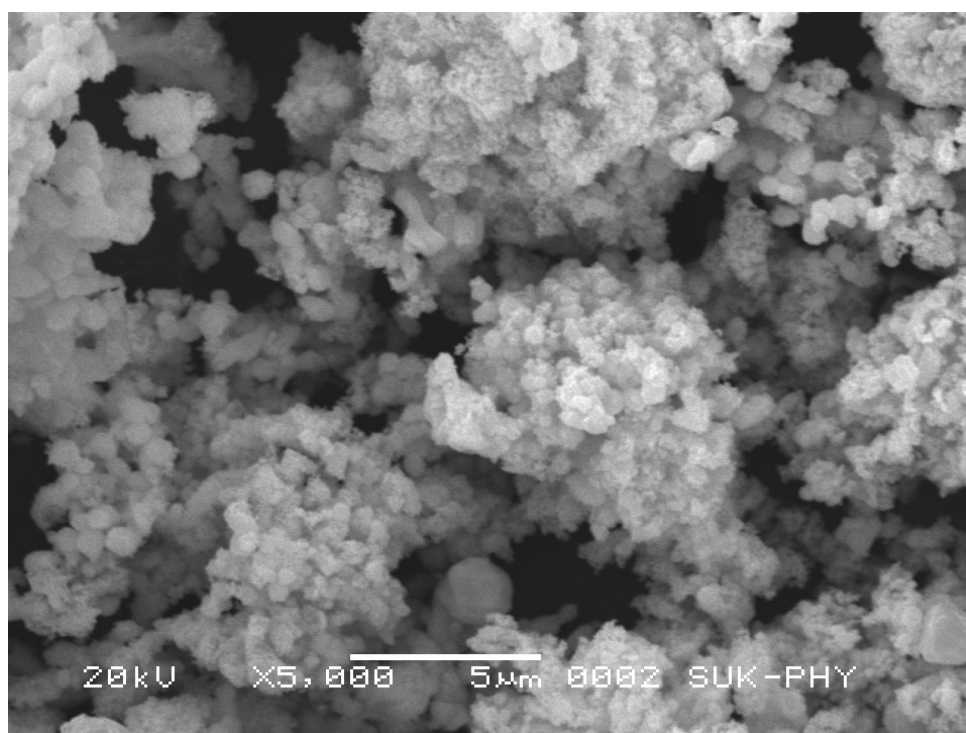


Figure 4: SEM image of Silver nanoparticles

TEM Analysis

TEM image shown in fig. 5 reveals that there is formation of poly-disperse spherical particles with non uniform distribution. For TEM measurements, a drop of solution containing the particle was deposited on a copper grid covered with amorphous carbon.

After allowing the film to stand for 2 minutes the extract solution was removed by means of blotting paper and the grid allowed drying before the measurement. It was observed that the nanoparticles formed were of different sizes and particle size was found to be 4.74nm, 8.17nm, 14.23nm and 18.98 and the mean size of about 11.5nm which lies in the nano range. The TEM measurement was done with JEOL model 1200Ex instrument operated at an accelerating voltage of 80kV. The TEM image was taken with very high resolution and MATLAB analysis gives the pixel depth of the image equal to 24bits and the image format as JPEG. The TEM Images have been taken from National Chemical Laboratory, Pune, India.

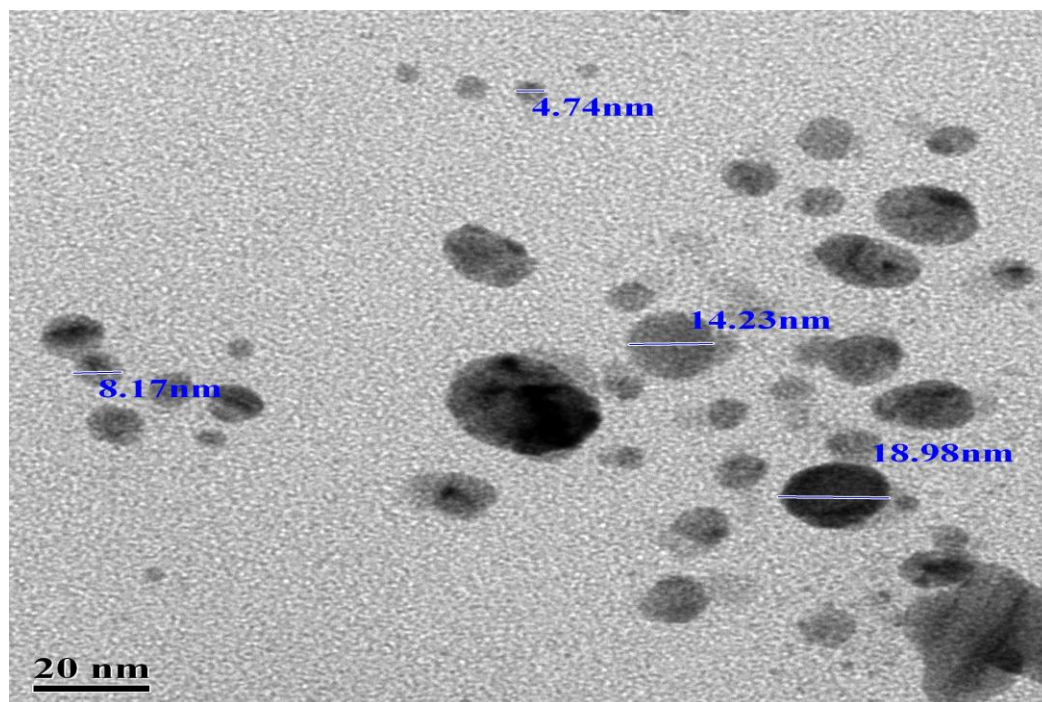


Figure 5: TEM Image for Silver showing size of nanoparticles

CONCLUSION

The present green synthetic method is a low cost approach and capable of synthesizing AgNPs at room temperature. The size and structure of obtained NPs were characterized by TEM, SEM, UV absorption, and XRD. Our results have shown that the plant leaf extract is the easy, economic and eco-friendly way to synthesize metallic nanoparticles. Moreover, this plant mediated synthesis method represents a considerable improvement in the preparation of AgNPs because of various advantages such as reduced reaction time, no need of capping agent, and better control over their size and shape. There are bulks of the investigations which have been carried at research laboratories in small scale whereas there are no reports on pilot plants or industrial scale fabrication of Nanomaterials using natural products. However, we believe that there are good opportunities for developing large scale synthesis using greener processes.

CONFLICT OF INTEREST

All Authors have no conflict of interest

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