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Green Synthesis and Optimization of Silver Nanoparticles from *Piper betel* and *Jatropha curcas* to Enhance α-Amylase Activity

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In the present study, the catalytic nature of silver nanoparticles was studied in the reaction mixture with amylase enzyme. Amylase is an industrially important enzyme that catalyzes the decomposition of starch into glucose. The silver nanoparticles in the study were synthesized using a green approach where the leaf extracts of *Piper betel* and *Jatropha curcas* act as reducing agent in reducing silver nitrate to silver nanoparticles. The reaction medium was optimized for better yield by altering the parameters like pH, temperature, silver ion concentration, reducing agent concentration and irradiation and hence the improvement in yield of silver nanoparticles was noticed. Moreover, the synthesized silver nanoaprticles were confirmed through UV-visible spectra, FTIR, SEM and EDX spectra. By optimizing, more stable particles were synthesized with a size of about 41 nm from both the leaf extracts. The synthesized nanoparticles were added in the reaction mixture with α-amylase and the reaction rate was enhanced to about three-folds in comparison with that without silver nanoparticles.

Keywords: Green synthesis, Silver nanoparticles, *Piper betel*, *Jatropha curcas*, α-Amylase.

INTRODUCTION

Currently more research is being carried out in the field of nanotechnology due to its size and unique properties. The metal nanoparticles are gaining its importance due to its optical, conductive and electrical properties. In this, silver nanoparticles find its application in various field as antimicrobial agent, catalytic, imaging, instrumentations and medical fields [1]. Inspite of these available techniques, we are looking into more convenient and economical way of synthesizing nanoparticles. Metal nanoparticles can be produced by different methods as physical, chemical and biological methods. Out of these methods, green synthesis of metal nanoparticles is of prime importance due to ecofriendly, economic and easy method of synthesis. In the mechanism of synthesis through plant routes the phyto-chemicals in the plant play a major role in the formation of metal nanoparticles through nucleation, growth and termination phase of syntheisis [2]. In the present study, green synthesis of silver nanoparticles were carried out from two different sources *viz*. *Piper betel* and *Jatropha curcas*. *Piper betel* and *Jatropha curcas* are known to be as excellent antioxidants and the nitrate reductase content is found to be relatively high [3,4]. However, drawback of using this green synthetic method is the low yield of nanoparticles. Through optimizing various parameters for the synthesis of silver nanoparticles, the yield of the nanoparticles can be increased to a considerable amount. Optimization enables rapid, stable and morphological synthesis of nanoparticles.

Among the metal based nanoparticles, silver nanoparticles have a great catalytic property due to its unique reactivity, selectivity, stability as well as recyclability in catalytic reactions [5]. In the current study, we have studied the catalytic nature of silver nanoparticles with biocatalyst, α-amylase. α-Amylase (EC 3.2.1.1) is an industrially important enzyme with high economic value that hydrolyses the α-1,4-linkage of starch molecule [6]. The synthesized and optimized nanoparticles were allowed to react with α-amylase.

EXPERIMENTAL

Preparation of plant extract: The leaf extract of *Jatropha curcas* and *Piper betel* were prepared using 10 g leaves thoroughly washed and finely cut, homogenized with 100 mL of distilled

water. The homogenized suspension was filtered and centrifuged to remove any suspended particles. The clear extract was refrigerated at 4 ºC for further use.

Synthesis of silver nanoparticles: Synthesis of nanoparticles was carried out by biological method. A 10 g each of *Jatropha curcas* and *Piper betel* leaves were washed thoroughly and then homogenized and filtered. A 10 mL of this extract was then added to 90 mL of distilled water. The setup was then incubated in sunlight under static conditions for 2 h.

UV-visible analysis: The reduction of pure Ag^+ ions was monitored by measuring the UV-vis spectrum of the reaction medium after diluting a small aliquot of sample with distilled water. The UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

FTIR analysis: The functional groups involved in the synthesis and stabilization of silver nanoparticles was studied by FTIR spectroscopy. The residual solution of 100 mL after reaction was centrifuged at 15000 rpm for 10 min and the resulting suspension was redispersed in 10 mL sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was analyzed using Bruker model Alpha ATR with wave number range from 400-4000/cm FTIR.

FESEM-EDS analysis: Thin film of sample was prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid (for membrane). A smear of nanoparticle sample was made, dried and coated with gold for ensuring conductivity using Carl Zeiss ultra 55 SEM with Oxford EDS detector.

Optimization of parameters in silver nanoparticles synthesis:

pH: In the present study, the pH was optimized for a wide range of pH from 2, 4, 6, 8, 10 and 12. The pH was adjusted using 0.1 N HCl and/or 0.1 N NaOH. The absorbance of the resulting solution was estimated spectrophotometrically.

Temperature: The optimum temperature for the reaction was estimated by experimenting at different temperatures from 0 to 100 ºC. The resulting solution was analyzed spectrophotometrically for its absorbance.

Time: The time of reaction was estimated by colour change after every 10 min for a period from 2 to 50 min. The conversion to AgNP′s was measured spectrophotometrically.

Pressure: The reaction mixture of silver nitrate and the leaf extract was exposed to varied pressures ranging from 5 to 15 psi using an 15 L Biogene autoclave. It was then compared with that of a reaction mixture maintained at atmospheric pressure.

Sunlight irradiation: The reaction mixture 1 mM silver nitrate was prepared. To a homogenized leaf extract solution of 10 mL of 1 mM $AgNO_3$ solution was added. The colour of the solution gradually changed from yellow to brown. The intensity of sunlight exposed measured by lux meter (Mextech LX-1010B) was 111,000 lux. The reduction of pure silver ions were monitored by measuring the optical density.

Microwave radiation: The reaction mixture was exposed to microwave radiation for a time period of 10 s to 40 s with a time interval of 10 s. The frequency of radiation exposed was 2450 MHz by Samsung 23L solo microwave oven.

Silver ion concentration: Various concentration of silver nitrate from 0.1 mM to 1mM was taken in order to find the optimum concentration for synthesis. The resulting solution was measured spectrophotometrically.

Concentration ratio of plant leaf extract and $AgNO_3$ solution: The concentration ratio of leaf extract and silver nitrate was optimized with the increase in concentration of leaf extract from 1 mL to 5 mL with 1 mM of $AgNO_3$ as constant. Ratio of leaf extract and $AgNO_3$ was optimized with the increase in the concentration of $AgNO_3$ from 0.1 mM to 1 mM solution in constant volume of leaf extract. The absorbance of the resulting solution was measured spectrophotometrically.

Reaction with α-amylase: The synthesized nanoparticles were then allowed to react with α-amylase (Himedia GRM638) with starch (Himedia GRM 3029) as an substrate. It was then compared with reaction without nanoparticles. The reaction rate was determined and comparison was drawn with reaction without nanoparticles.

RESULTS AND DISCUSSION

Visual characterization: The colour change in the reaction mixture was recorded through visual observation. The colour change from yellow to dark brown indicated that AgNPs were synthesized (Fig. 1) successfully. The colour change observed is due to the reduction of silver ions present in $AgNO_3$ solution.

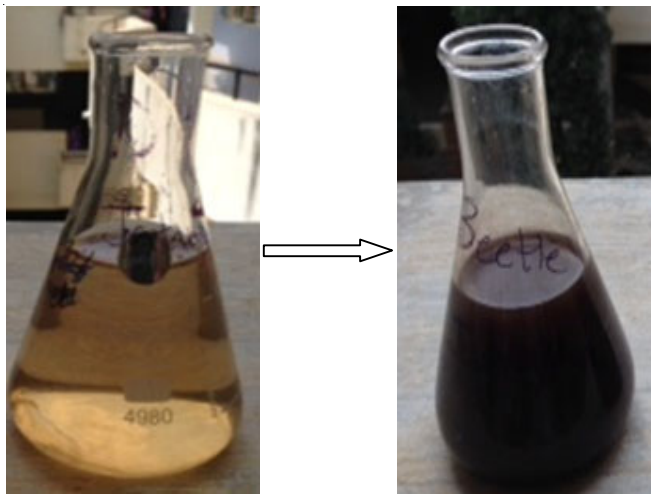

Fig. 1. Colour change of plant extract from yellow to dark brown

UV-Vis spectral analysis: Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of colour change. The colour change is due to the surface plasmon resonance phenomenon (SPR). The useful nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The characteristic peaks were observed around 438 nm in case of *Piper betel* whereas the bands for *Jatropha curcas* were observed around 430 nm [7] (Fig. 2). The reduction of the metal ions occurs fairly rapidly. The metal particles were observed to be stable in solution after their synthesis. By stability, we mean that there was no observable variation in the optical properties of nanoparticle solutions with time.

FTIR analysis: The FTIR spectra (Fig. 3) depicted the possible biomolecules present in the leaf extract, which are

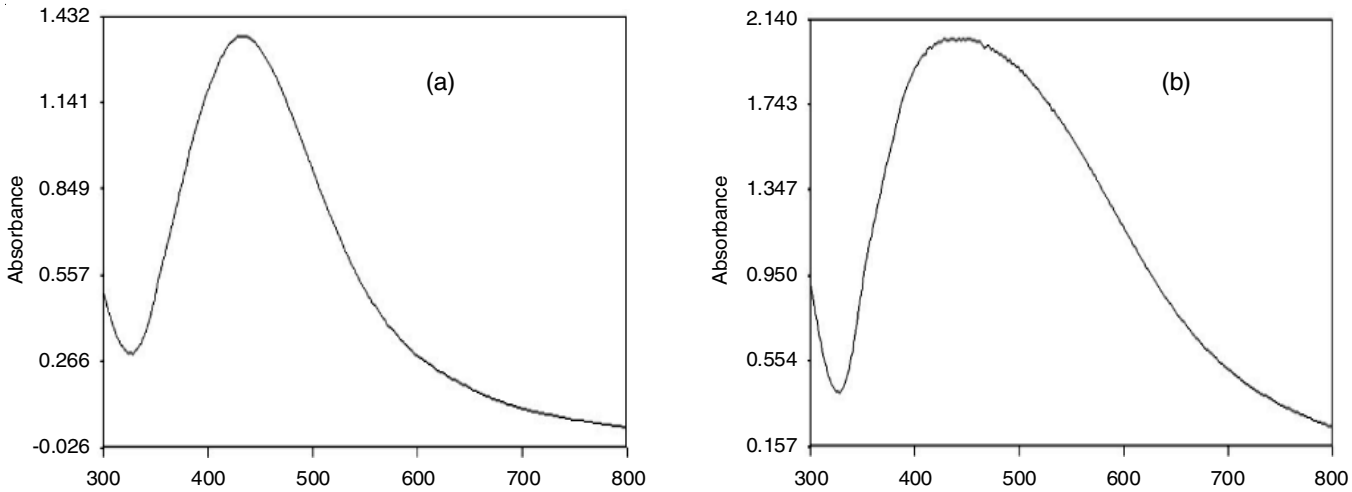

Fig. 2. UV-vis spectral analysis of (a) *Jatropha curcas* AgNPs and (b) *Piper betel* AgNPs

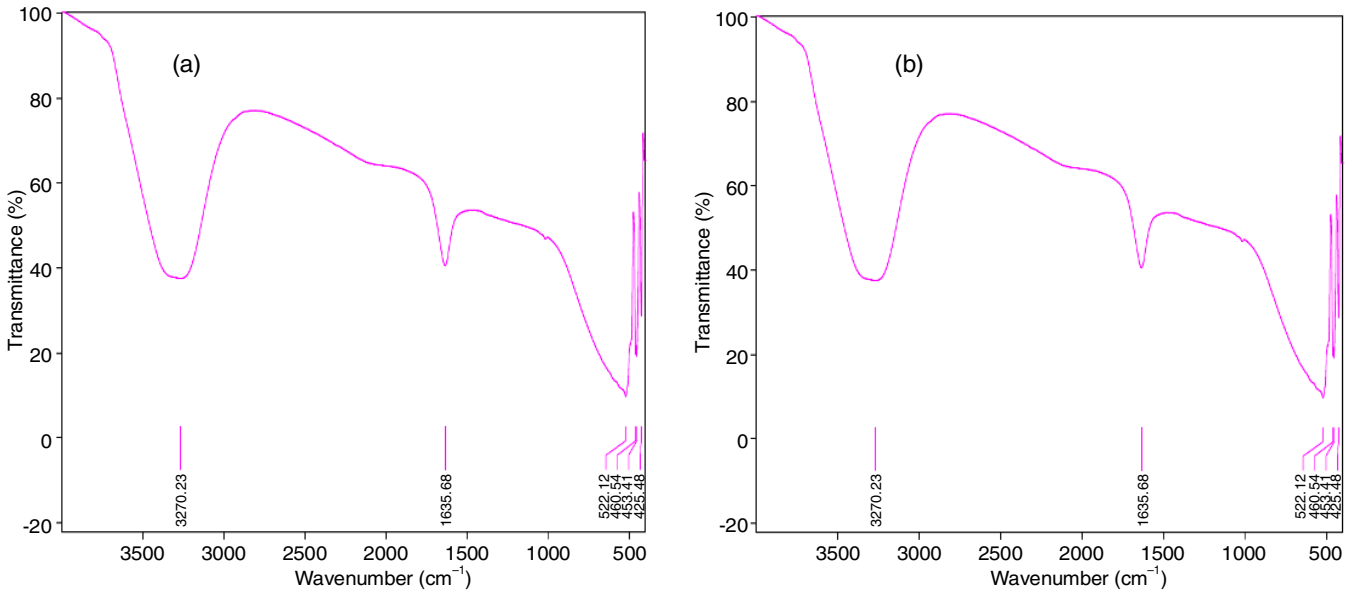

Fig. 3. FTIR analysis of (a) *Jatropha curcas* AgNPs and (b) *Piper betel* AgNP

accountable as the reducing agents for silver ions and their interaction with AgNPs. The peak at 3270.23 cm^{-1} indicates C-H stretching due to alkynes. A band at 1635.68 cm^{-1} (C=C) stretching interactions to alkane due to the metabolites present in leaf extract. The synthesized silver nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups. It is also confirmed that the carbonyl groups from amino acid residues and proteins has the stronger ability to bind metal indicating the proteins could possibly form metal nanoparticles (*i.e.*, capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium [8]. The biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium.

FESEM-EDS analysis: SEM analysis gives the size and shape of nanoparticles produced. Average particle size of silver nano-particles from *Jatropha curcas* was found to be 42 nm whereas from *Piper betel*, it was found to be 41 nm. The nanoparticles produced were of uniform size and spherical in shape (Fig. 4). The EDS spectrum of spherical nanoparticles prepared with the bioreduction method showed the peaks around 2.7 keV corresponding to the binding energies of AgCl. The results indicated that the reaction product was composed of high purity silver nanoparticles.

Fig. 5 shows the EDX spectrum of silver nanoparticles synthesized by using sources as (a) *Piper betel* leaf extract and (b) *Jatropha curcas* leaf extract. The concentration of elemental silver was found to be 56.68 and 69.12 %, repectively in *Piper betel* and *Jatropha curcas*.

Optimization parameters for AgNPs synthesis

Effect of pH: The pH has also affected the synthesis of silver nanoparticles. The reducing agent, nitrate reductase would have undergone structural change as its activity was seen maximum at pH 10 which reduces further (Fig. 6). The optimum pH for the nanoparticle synthesis was observed at pH 10.

Effect of pressure: The rate of synthesis of silver nanoparticles was checked at varied pressures of 5, 10 and 15 psi. It was observed that below 5 psi, there was no change. However, the rate of synthesis increased from 10 psi onwards. After 15 psi, the

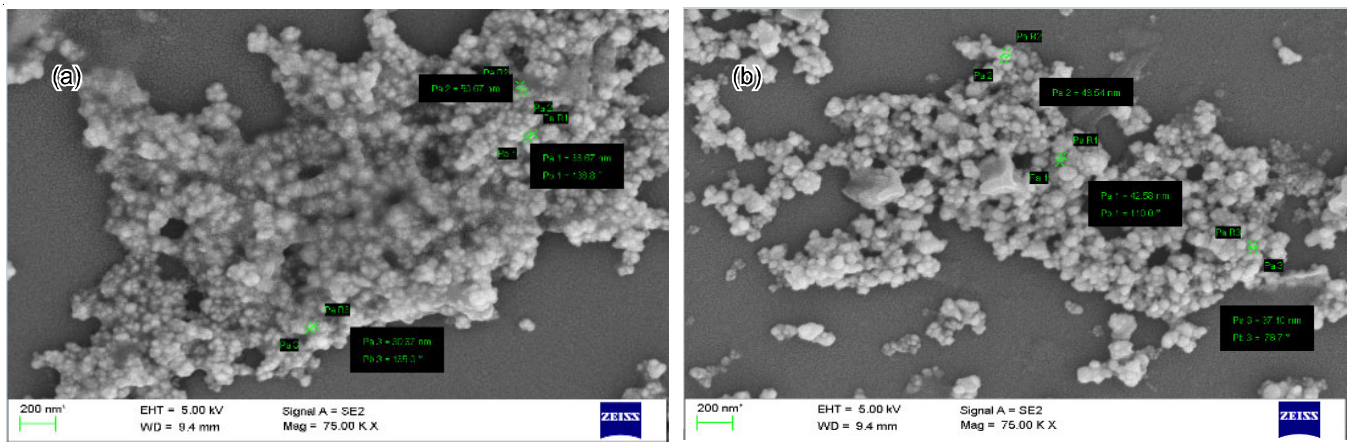

Fig. 4. Electron microscope of AgNPs (a) from *Piper betel* (b) from *Jatropha curcas*

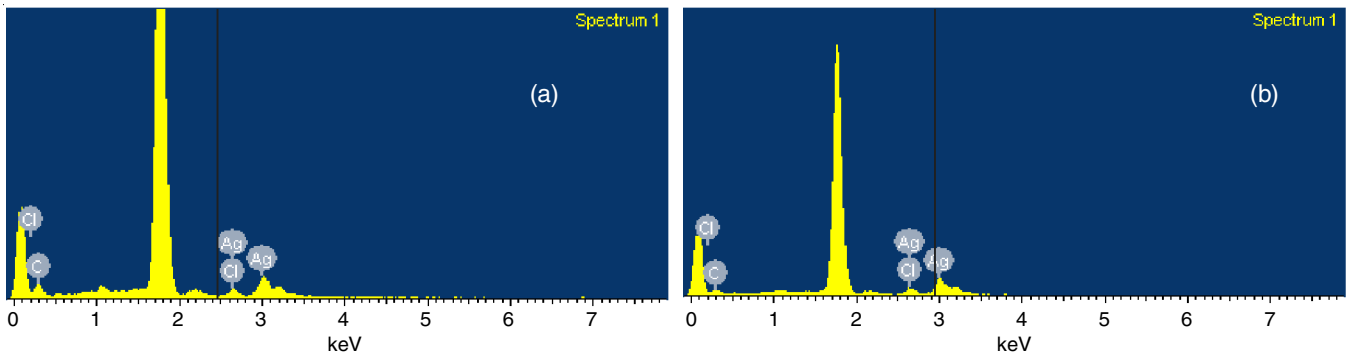

Fig. 5. EDX of silver nanoparticles synthesized from (a) *Piper betel* (b) *Jatropha curcas*

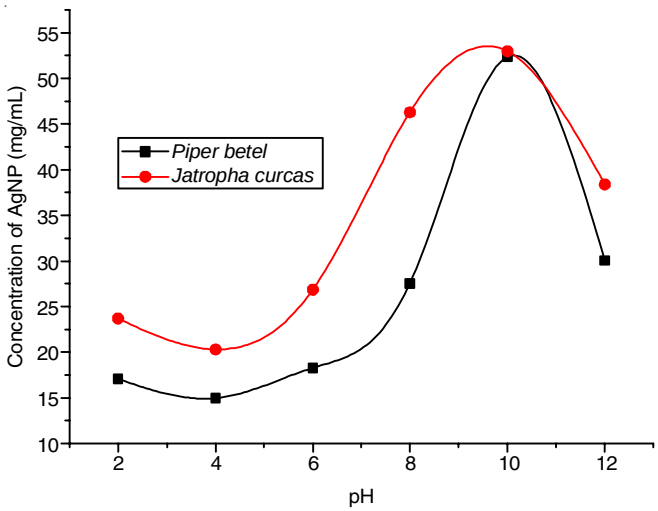

Fig. 6. Effect of pH on *Piper betel* and *Jatropha curcas* extracts

rate of synthesis kept on increasing. The study was carried out till 15 psi because the autoclave used in the present experimental studies and had maximum operational pressure of 15 psi (Fig. 7).

Effect of microwave: Microwave irradiation has influenced the silver nanoparticle synthesis in a very less time. It was observed that for an exposure of 10 s to microwave radiation has initiated the nanoparticle synthesis to about three-folds higher than at normal conditions. And beyond the exposure of 40 s, reaction mixture completely reduced the rate of reduction was observed to be four-folds high and reached saturation, whereas the reduction activity had not yet started in the reaction mixture under normal conditions (Fig. 8).

Effect of silver ions concentration: Different concentrations of $AgNO_3$ were utilized in order to maximize the rate of AgNPs production. The maximum yield of AgNPs when the concentration of $AgNO_3$ solution was 1 mM which is the optimum concentration for silver nanoparticle synthesis. Below this concentration, the yields not much significant changes were observed paving a way of even using lesser $AgNO_3$ solution for synthesis (Fig. 9).

Effect of plant extract and silver ion ratio: The different concentrations of plant extracts used showed varying absorption. The maximum absorption was observed at the ratio of 1:4 of plant extract with that of $AgNO_3$ (Fig. 10). Reducing the concentration of plant concentration will minimize the contamination and hence synthesizing pure AgNP.

Effect of temperature: The temperature of reaction medium determines the nature of the nanoparticles formed. In general, temperature elevation increases the rate and efficiency of nanoparticle synthesis. The temperature of the reaction increased the rate of formation of AgNPs. On elevating the temperature, it was found that the rate of synthesis has increased as the collision of molecules are better at elevated temperature [9] (Fig. 11).

Effect of time: The rapid rate of synthesis was observed immediately after the addition of reducing agent. It was observed that by increasing the time of reaction the reaction peak was increased and more AgNPs were formed during the incubation time range of 0-10 min. After 10 min, there was very gradual change in absorption indicating the stability of AgNPs colloidal solution [10]. The optimum rate of synthesis for *Piper betel* and *Jatropha curcas* leaf extracts were observed at 10 min (Fig. 12).

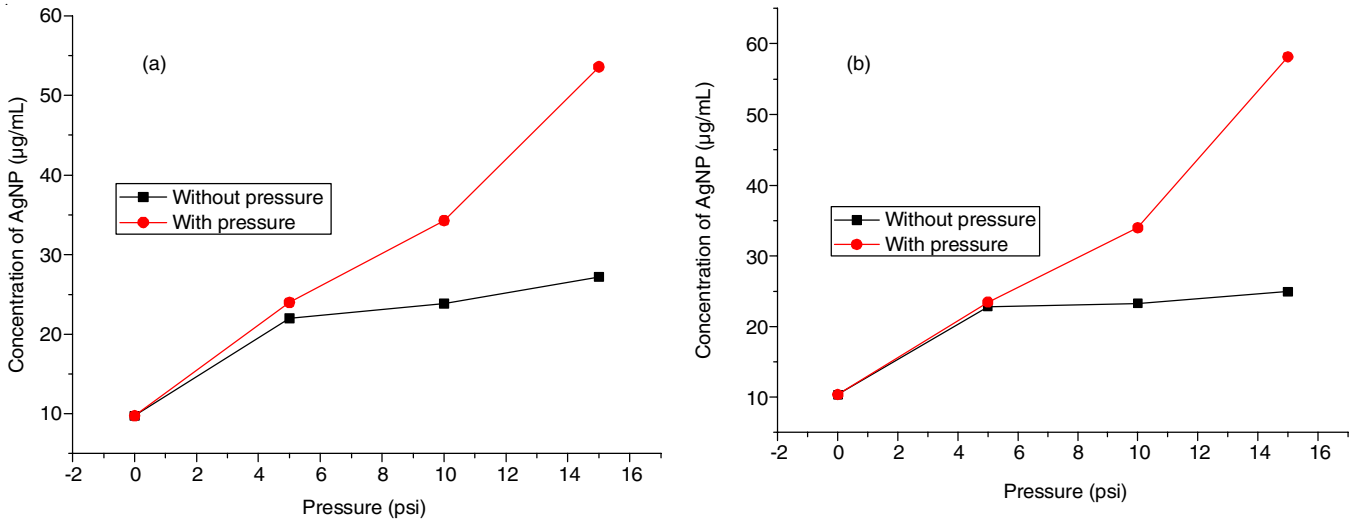

Fig. 7. Comparison of effect of pressure and without pressure on (a) *Piper betel* extract and (b) *Jatropha curcas* extract

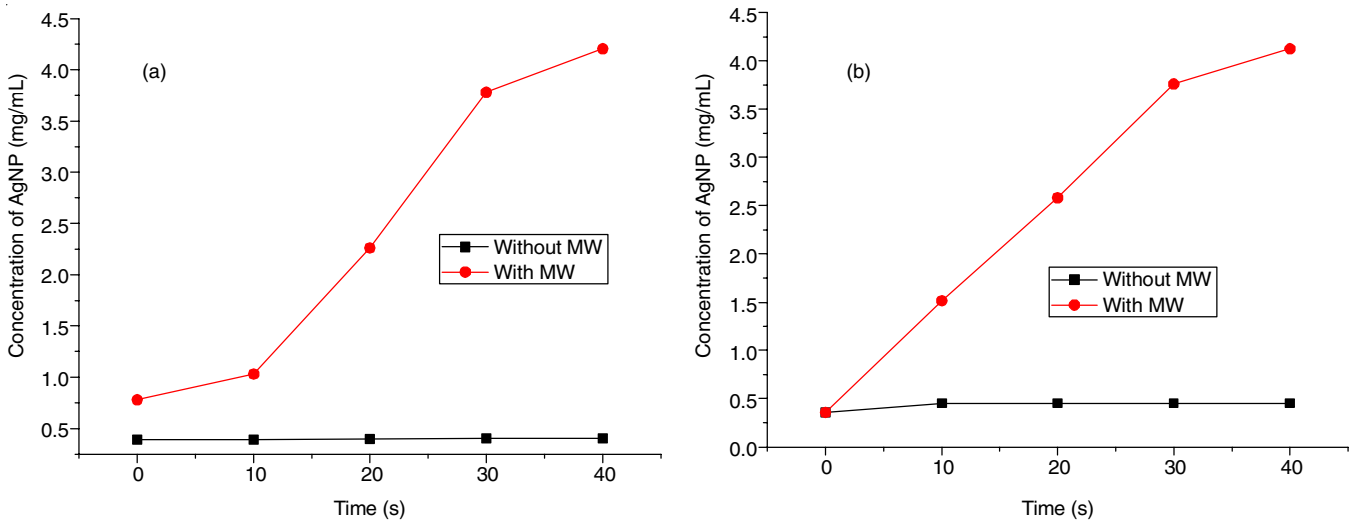

Fig. 8. Comparison of with and without microwave radiation on (a) *Jatropha curcas* extract and (b) *Piper betel* extract

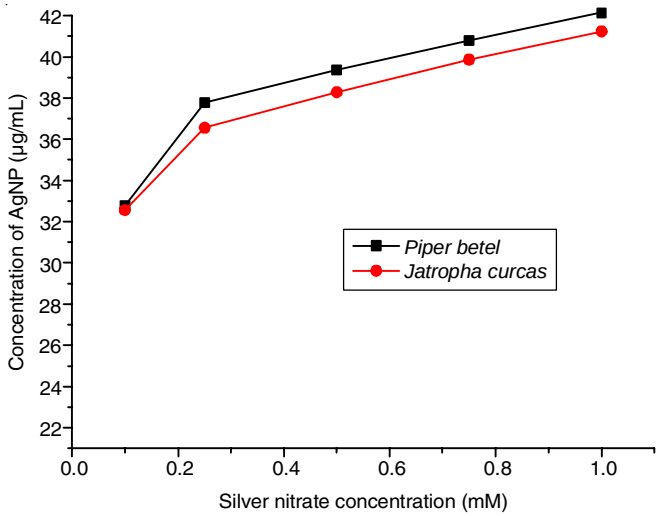

Fig. 9. Effect of silver ion concentration on *P. betel* and *J. curcas* extracts

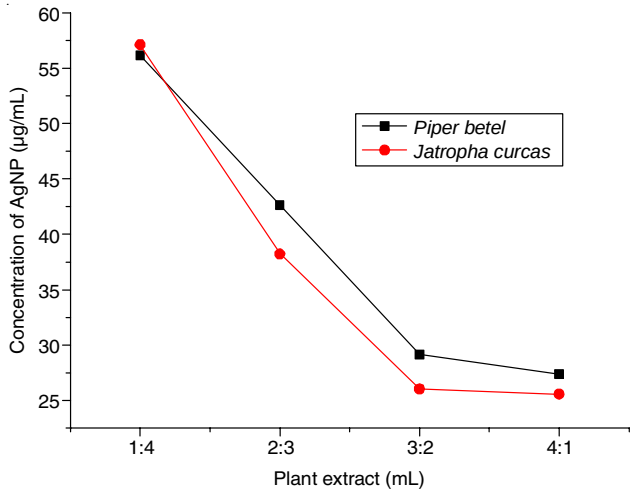

Fig. 10. Effect of plant extract on *Piper betel* and *Jatropha curcas* extracts

Effect of sunlight: During the synthesis of nanoparticles the reaction mixture were exposed to sunlight. The colour of the reaction mixture turned to dark brown immediately after exposing to sunlight indicating the rate of synthesis of silver nanoparticle was high. On comparison of the reaction with the dark it is observed that the sunlight has enhanced the reaction to about three-folds (Fig. 13) more thus concluding the sunlight acts as a catalyst during the reduction reaction during the synthesis of silver nanoparticle [11].

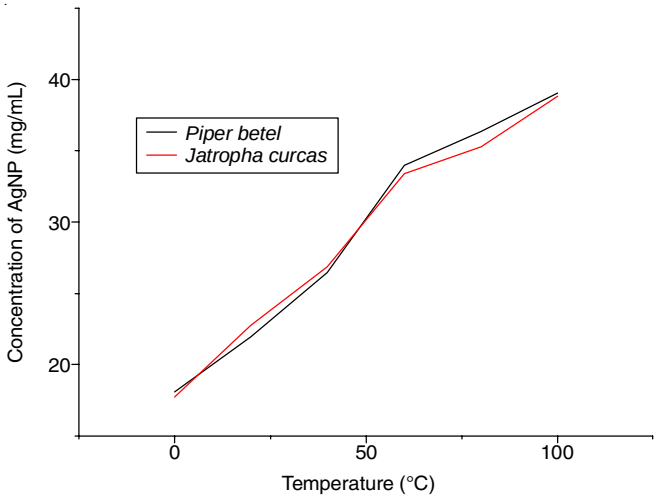

Fig. 11. Effect of temperature on *Piper betel* and *Jatropha curcas* extracts

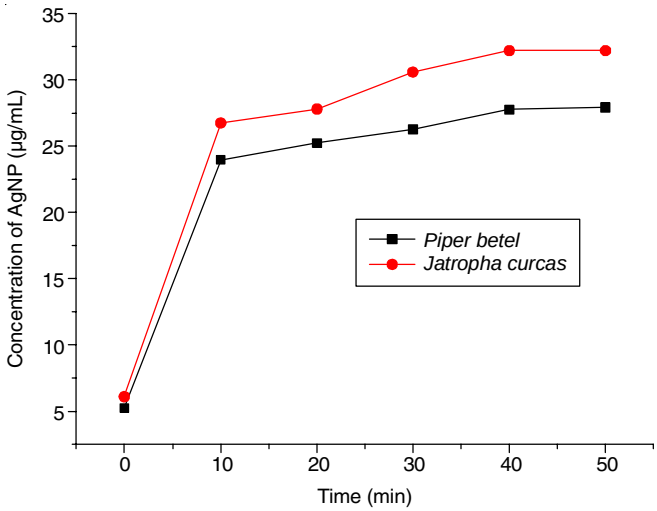

Fig. 12. Effect of time on *Piper betel* and *Jatropha curcas* extract

α-Amylase activity: The interaction of silver nanoparticles with α-amylase enzyme was studied by DNS assay. The activity of enzyme with and without nanoparticles was studied. The reaction rate constant value was found to be 3.837×10^{-3} s^{-1} for the activity without nanoparticles and with nanoparticles, was found to be 9.457×10^{-3} s^{-1}. It is clearly seen the addition of silver nanoparticles has increased the reaction rate to about three-folds (Fig. 14). Thus proved that silver nanopartilces can act as a potential biocatalyst [12] and thereby reducing the utilization of enzymes in industries.

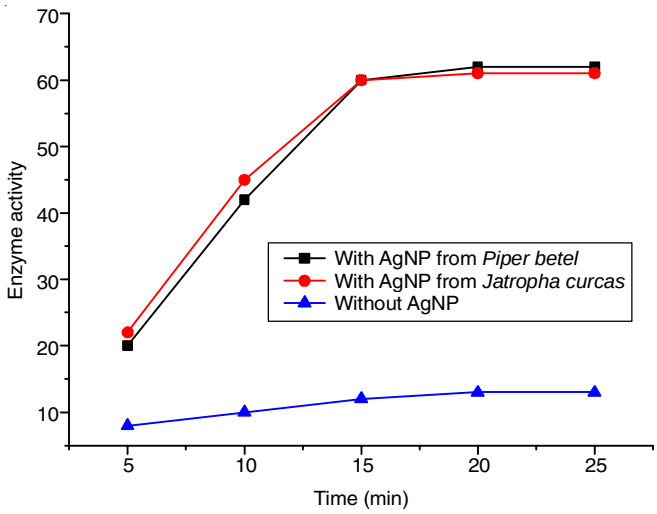

Fig. 14. Comparison enzyme activity of α-amylase with and without nanopartricles

Conclusion

The green synthesis enabled a more ecofriendly way of synthesizing silver nanoparticles from *Piper betel* and *Jatropha curcas* leaf extracts. Though the biological method is a slow reduction reaction, the reaction can be enhanced by optimizing various such as temperature, pH, pressure, silver nitrate concentration, plant extract volume and influence of radiation as sunlight, microwave, *etc*. In comparison with all the optimization process exposure to irradiation by microwave has shown the fastest method of optimizing. The size of the synthesized silver nanoparticles from both leaf extracts were same thus indicating that at optimum condition all the metabolites have contributed to the synthesis of the nanoparticle and the complete reaction has taken place. The optimized silver nanoparticles were then allowed to interact with α-amylase as a catalyst for the enzyme for starch as substrate. It is seen that the enzyme activity was found to increase three-folds.

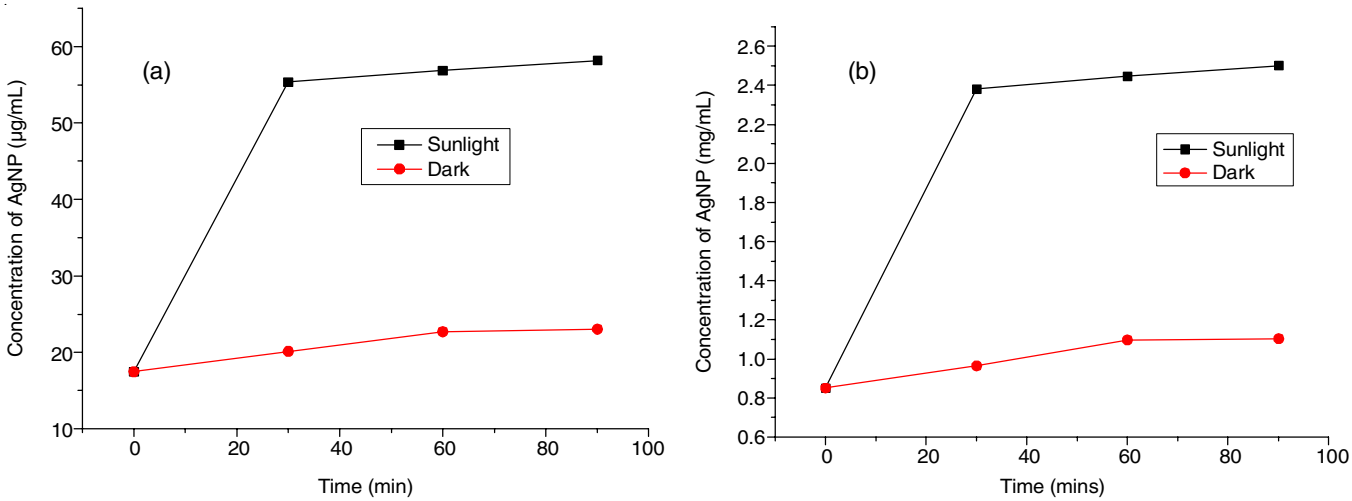

Fig. 13. Comparison of sunlight and dark on (a) *Piper betel* and (b) *Jatropha curcas* extract

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. N. Khatoon, J.A. Mazumder and M. Sardar, *J. Nanosci. Curr. Res.*, **2**, 107 (2017); https://doi.org/10.4172/2572-0813.1000107.
2. M.J. Firdhouse and P. Lalitha, *J. Nanotechnol.*, **2015**, Article ID 829526 (2015); https://doi.org/10.1155/2015/829526.
3. K. Ghosh and T.K. Bhattacharya, *Molecules*, **10**, 798 (2005); https://doi.org/10.3390/10070798.
4. N.S. Tomar, M. Sharma and R.M. Agarwal, *Physiol. Mol. Biol. Plants*, **21**, 83 (2015); https://doi.org/10.1007/s12298-014-0272-0.
5. Z.-J. Jiang, C.-Y. Liu and L.-W. Sun, *J. Phys. Chem. B*, **109**, 1730 (2005); https://doi.org/10.1021/jp046032g.
6. A. Sundarram and T.P.K. Murthy, *J. Appl. Environ. Microbiol.*, **2**, 166 (2014); https://doi.org/10.12691/jaem-2-4-10.
7. P. Mulvaney, *Langmuir*, **12**, 788 (1996); https://doi.org/10.1021/la9502711.
8. B.D. Prasad Raj, K. Mallikarju, G.R. Dillip, G. Narasimha and N.J. Sushma, *Res. J. Nanosci. Nanotechnol.*, **2**, 17 (2012); https://doi.org/10.3923/rjnn.2012.17.23.
9. H. Korbekandi, Z. Ashari, S. Iravani and S. Abbasi, *Iran. J. Pharm. Res*., **12**, 289 (2012).
10. S.S. Birla, S.C. Gaikwad, A.K. Gade and M.K. Rai, *The Scientific World J.*, **2013**, Article ID 796018 (2013); https://doi.org/10.1155/2013/796018.
11. A.J. Das, R. Kumar and S.P. Goutam, *J. Bioeng. Biomed. Sci.*, **6**, 208 (2016); https://doi.org/10.4172/2155-9538.1000208.
12. V. Ernest, P.J. Shiny, A. Mukherjee and N. Chandrasekaran, *Carbohydr. Res.*, **352**, 60 (2012); https://doi.org/10.1016/j.carres.2012.02.009.