Optimization of process parameters for the green synthesis of silver nanoparticles using Plackett-Burman and 3-level Box–Behnken Design

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Abstract

Background:

The present work describes the synthesis of silver nanoparticles (AgNps) from leaf extracts of *Piper betle* and *Jatropha curcas* using a green approach. Green synthesis of nanoparticle is superior over other methods of synthesis as it is ecofriendly and cost effective. The phytochemical components in the leaf extract play a vital role in reducing the AgNO_3 and hence synthesizing the silver nanoparticles. During this reduction activity, the several factors which affect the synthesis of nanoparticles were investigated and optimized according to the yield of nanoparticles. The experimental conditions investigated were pH, temperature, pressure, time of reaction, microwave radiation, UV radiation and concentration of plant extract, silver nitrate & sunlight. Satisfactory yields of silver nanoparticles synthesis from plant extract were obtained by using optimum conditions when compared to conventional synthesis of nanoparticles. The optimization parameter was later

Result:

A mathematical model was formulated to correlate the interactive influence of the parameters and the significant reduction. Plackett-Burman design (PBD) indicated that concentration of plant extract, concentration of silver nitrate and sunlight were the major parameters affecting the synthesis of silver nanoparticle. The mutual interactions of these variables are mapped in the design by 3 Box-Behnken design (BBD). The significant factors and their interactions in the green synthesis were examined by analysis of Variance (ANOVA). The result indicated that the BBD was a good predictive model for the experimental results. Though the plant extracts are different, the characterization of the synthesized nanoparticle after optimization of parameters showed uniform size and shape i.e. spherical shape and size of 41 nm.

Conclusion:

The silver nanoparticles were synthesized under different optimization parameters were uniformly shaped (spherical) and sized (41 nm) particles. The mathematical models Plackett-Burman and Box-Behnken helped in analyzing the impact of the optimization parameters.

Background

Nanoparticles are those of 1-100 nm size and are increasing attention due to wide applications in the areas of science and technology. Metal nanoparticles are gaining more importance due to their optical, electrical and catalytic properties. They are widely applied in various industries as medicine, water treatment, energy conversion, magnetics, mechanics and so on [1, 2]. Although there are various synthesis methods of Silver nanoparticles as chemical reduction [3], micro emulsion [4], photoreduction [5] and radiation [5]; green synthesis of AgNps serves as a more economical and eco friendly way of synthesizing. Though many physical, chemical and biological methods have been researched for nanoparticle synthesis, silver nanoparticles synthesized via physical and chemical methods cannot avoid the use of toxic agents. These methods rely on use of organic solvents and toxic chemical as N,N-dimethylformamide, sodium borohydride, and hydration hydrazine [6]. Green synthesis seems as an alternative ecofriendly and sustainable approach in synthesizing AgNps. Although the technology is proven to be cost effective it also takes into account environmental safety. The phytochemical components in the leaf extract: asterpenoids, flavones, ketones, aldehydes, amides and carboxylic acid play a vital role in the reducing activity of the silver nitrate solution. The secondary metabolites found in the plant extract as flavones, organic acids and quinines are water soluble metabolites and are responsible for its immediate reduction of ions. Green synthesis method thus enables better synthesis by fast reductions of ions. [7] However by optimizing various parameters as temperature, pH more stable and size controlled nanoparticles can be synthesized. The optimization of synthesis through statistical method is more effective when compared with classical. [8]

The literature says that the extracts of various plants have been employed for the synthesis of silver nanoparticles like Neem leaf [10], *Aloe vera* [11], xerophytes (*Bryophyllum sp.*), mesophyte (*Cyperus sp.*), hydrophyte (*Hydrilla sp.*) [12], *Gliricidia sepium*, [13], *Rosa rugosa* [14], *Chenopodium album* [15], *Cycas* leaf [16], *Acalypha indica* [17], *Cassia fistula* [18], *Hibiscus rosasinensis* [19], weeds as *Ipomoea aquatica*, *Enhydra fluctuans* and *Ludwigia adscendens* [20], *Psidium guajava* [21], mangosteen leaf [22], *Ocimum sanctum* [23], *Cocos nucifera* coir extract [24], etc. It is observed that comparatively microorganisms mediated synthesis of metallic nanoparticles are faster to plant mediated synthesis. Plant metabolites had shown faster rate of reduction of metal ions and produces stable metallic nanoparticles. By using plant extracts for the synthesis of nanoparticles, the shape and size of the nanoparticles can be controlled and altered by modifying certain conditions as pH and temperature [25]

In the present work, the green synthesis of silver nanoparticles is carried out from two sources namely, *Piper betle* and *Jatropha curcas*. The various factors affecting the synthesis of silver nanoparticles as concentration of AgNO_3 , concentration of plant extract, pH, temperature, time, microwave radiation, UV Radiation exposure, sun light exposure reaction, volume and pressure, etc. were studied. The optimization of these parameters was carried out by Plackett-Burman design to study the effect of these variables in the synthesis of silver nanoparticles. During synthesis of silver nanoparticles preliminary individual experiments were carried out to establish accurate range of process variables considered for optimization. These parameter ranges were screened for the best three significant factors by Plackett-Burman design (PBD). PBD is a very efficient design when only main effects are of interest. They use the minimum number of runs to quickly identify the factors with a significant effect on the response. Further three significant factors were selected using PBD, and then the significant variables were further optimized by Box-Behnken design (BBD) to determine the optimum levels of these optimized parameters to have a high yield of silver nanoparticles from plant extracts. BBD are of advantage in addressing the issue of where the experimental boundaries should be and in particular to avoid treatment conditions that are extreme. Two statistical tools, Plackett-Burman design (PBD) combined with Box-Behnken design (BBD) offered efficient and effective process mode for experiments. The optimized conditions were then used for synthesis of silver nanoparticles and it was observed that uniform size and shaped nanoparticles were obtained.

Results And Discussion

Green synthesis of Silver nanoparticle:

The reduction activity of Ag^+ to Ag^0 is shown by the color change from light green to brown color. The concentration is calculated by a calibration graph measuring the optical density at 430 nm. Figure 1 a) shows the plant extract of yellow color and b) shows the change of color to brown showing the reduction activity of the plant extract to silver nanoparticles. The phytochemical components present in the leaf extracts enable a quicker reduction of ions when compared to other biological methods. Flavones, organic acids and quinines present in the leaf extract are water soluble phytochemicals responsible for immediate reduction of Silver ions. These were confirmed through the phytochemical analysis and was found that in *Piper betle* Flavones were 32.5 mg/g, organic acids 8%(w/w) and quinines about 43 mg/g. Similarly, the phytochemical components in *Jatropha curcas* Flavones were 37 mg/g, organic acids 12% (w/w) and quinines about 39 mg/g.

Optimization of Silver nanoparticle:

The optimization parameters influencing the silver nanoparticle synthesis were analyzed using Plackett burman design (PBD) and Box Behnken Design (BBD). The results obtained through the preliminary experiments were analyzed using statistica software.

Plackett-Burman design (PBD):

Plackett-Burman experimental design is most simple, less time consuming and well-established system for screening different variables and selection of most significant variable and conditions for better results. In our study, a total of 9 independent variables were screened using Plackett-Burman experimental design (Table 1).

The experiment was conducted in 12 runs to study the effect of the selected variables on the synthesis of silver nanoparticles. (Table 2). In present study, Plackett-Burman experimental design showed a markedly wide variation in yield of silver nanoparticles :19.57-41.53 $\mu\text{g/mL}$ in *Piper betle* extract and 20.46-41.51 $\mu\text{g/mL}$ in *Jatropha* extract. This variation is mainly due to the effect of different optimization parameters on the synthesis of Silver nanoparticles and it has reflected the importance of optimization of variables to increase the yield of silver nanoparticles. The maximum yield of 41.53 $\mu\text{g/mL}$ from Piper Betel extract and 41.51 $\mu\text{g/mL}$ from *Jatropha* extract was achieved in the run number 1, while the minimum yield of 20.46 $\mu\text{g/mL}$ from *Piper betle* extract and 19.57 $\mu\text{g/mL}$ from *Jatropha* extract were observed in the run number 12. The Run1 has X_1 , X_3 , X_7 , and X_8 as the high value parameters and X_2 , X_4 , X_6 and X_{10} as low value parameters. The results show that pH, Pressure, concentration of plant extract and silver nitrate had a greater impact on nanoparticle synthesis. The plant extract has the reducing agent and the silver nitrate has the silver ion which is a major component for the silver nanoparticle synthesis. Pressure is also a contributing factor as increase in pressure influence the dynamic movement of the molecules resulting in more interaction and thereby synthesizing more nanoparticles. Change in pH contributes the H^+ ions there by changing the conformation of the reducing agent and hence allowing increasing the yield of nanoparticles. Also the Run 12 has the minimum yield on silver nanoparticle and all the parameters were kept at low level (-1) at this trial.

Table 3 show the effect of each independent variable on the silver nanoparticles synthesis. The values shows the determination of the effect of each variable and a large numerical value either positive or negative indicates that a factor has a large impact on synthesis; while an effect close to 0 means that a variable has little or no effect. With respect to the table we can see that seven variables out of nine namely, concentration of AgNO_3 , pH, temperature, time, microwave radiation, sun light exposure have positive affect whereas other two variables namely, concentration of plant extract and UV radiation exposure. Regression coefficients estimate the unknown parameters and describe the relationship between a predictor variable and response. The sign of each coefficient indicates the direction of the relationship between a predictor variable and the response variable. Standard error is a measure of the statistical accuracy of an estimate, equal to the standard deviation of the theoretical distribution of a large population of such estimates. Main effect is the effect of one independent variable on the dependent variable. It ignores the effects of any other independent variables. P-value suggest us the significance of the parameter for the synthesis of nanoparticle

The Pareto chart shows the order of effects of nanoparticles synthesis (Figure 4). The length of each factor is proportional to the absolute values of the estimated effects. Therefore these terms are supporting hierarchy. The final decision on the most effective parameters requires the assessment of half-normal probability plot. Furthermore the positive and negative signs showed that the response is improved from the low to high level or not.

The Pareto chart shows 7 significant and 2 non-significant variables (Table 3) affecting the synthesis of silver nanoparticles. The leaf extract was the most significant variable with p value = 0.04 (*Piper betle*) and 0.0002 (*Jatropha curcas*) as leaf extract has the major reducing agent in it, followed by silver nitrate with p-value = 0.04 (*Piper betle*) and 0.0003(*Jatropha curcas*) as silver nitrate contribute the silver ions for nanoparticle synthesis, sunlight exposure with p-value = 0.07 (*Piper betle*) and 0.001(*Jatropha*) as sunlight serves as a photocatalyst for the reduction of silver ions ,pH with p-value = 0.015 (Betel) and 0.011(*Jatropha curcas*) as pH changes the structure of the reducing agent , Pressure with p-value = 0.19(*Piper betle*) and 0.003(*Jatropha curcas*) and it influences the rate of , UV radiation with p-value = 0.20(*Piper betle*) and 0.0005(*Jatropha curcas*) , MW radiation with p-value 0.47(*Piper betle*) and 0.01(*Jatropha curcas*) as it can proceed with the reduction activity with minimum time, Temperature with p-value = 0.57(*Piper betle*) and 0.502(*Jatropha curcas*) as temperature increases the rate of interaction but the reducing agent may degrade at higher temperatures, and time with p-value =0.86(*Piper betle*) and 0.0095(*Jatropha*), as time increases the synthesis of silver nanoparticles increases and after a while attains saturation due to non availability of silver ions or the reducing agent.

Table 4 is on ANOVA analysis and F-test. Analysis of variance (ANOVA) is used to determine whether there are any statistically significant differences between the means of three or more independent variables. ANOVA uses F-tests to statistically test the equality of means. The degrees of freedom associated with SSR will always be 1 for the simple linear regression model. The values from the ANOVA table shows that they are statistically significant.

Table 5 shows the observed and predicted values of Silver Nanoparticles synthesis from the two leaf extracts *Piper Betle* and *Jatropha curcas*. The half normal probability plot was also used to identify the significant parameters. In this plot, The factors having effects near the straight line through zero are not significant while those deviating from the straight line are significant.[28] Figure 3 shows the half normal plot, can be used to which the algorithms of Length and Dong are applied to identify significant effects and to estimate standard deviation of the effects. Significant effects in half normal plots are detected through visual inspection (29). The slope of the line through the effects assumed to be non-significant gives an estimate of the standard deviation (σ) of the error. From Figure 2 it can be concluded that plant extract, Concentration of Silver Nitrate solution and sunlight has greater impact on AgNp synthesis. The silver nitrate solution serves as the major donor of the silver ion contributing for the synthesis of silver nanoparticles. Sunlight act as a photo catalyst in the reduction of silver ion. The plant extract has protein, nitrate reductase which acts as an reducing agent in the reduction process[30]. Therefore, at high and low pH, less accumulation of nanoparticles may occur due to the aggregation of the protein structure [31,32] The three parameters (plant extract, Concentration of Silver Nitrate solution and sunlight) which have a greater influence on the silver nanoparticle synthesis were selected for Box Behnken design.

Box Behnken Design (BBD):

A Box-Behnken experimental design with 3 independent variables at 3 different levels, low (-1), medium (0) and high (+1) was used to study the effects on dependent variables. A Box-Behnken experimental design has the advantage of requiring less experiments (15 batches) than would a full factorial design (27 batches). Table 6 shows the Box–Behnken experimental design and yield of silver nanoparticle obtained through experimentation.

The results of ANOVA analysis are depicted in Table 7. The F value, p-value, MS value indicate that the relation between the response and the selected parameters is statistically significant [33]. The adequacy of the model developed was evaluated based on the correlation coefficient R^2 and standard deviation value [34]. The closer the R^2 value of unity and smaller the standard deviation implying more accurate response and repeatability that could be predicted by the model. The R^2 and R^2 -(adj.) for *Piper betle* were obtained as 1 and 1 whereas for *Jatropha curcas* were obtained as 1 and 0.9999 respectively. The compatibility of R^2 to R^2 means a good adaptation of the theoretical values for the experimental data of the model [35]. The model can be considered as a practical model for the prediction of the factors within the tested ranges.

By applying multiple regression analysis on the experimental data, the following second order polynomial equation was found to explain the silver nanoparticle synthesise from piper betel (3) and *Jatropha curcas* (4) plant extract by only considering the significant terms and is shown below:

$$Y = 35.4116 + 20.5793 X_1 - 16.4444 X_1^2 + 38.9287 X_2 - 12.8528 X_2^2 - 0.2572 X_3 + 0.0023 X_3^2 - 57.9182 X_1 X_2 \quad (3)$$

$$Y = 48.9731 - 83.4124 X_1 + 112.5000 X_1^2 + 2.9125 X_2 + 0.2653 X_2^2 + 0.1811 X_3 - 0.0011 X_3^2 - 0.0082 X_1 X_2 - 0.0011 X_1 X_3 + 0.0002 X_2 X_3 \quad (4)$$

The value of the correlation coefficient (R^2) of Equation (3) and (4) was found to be 0.99 and 1 respectively, indicating a very good fit. The results clearly indicate that the Y (Yield of Silver nanoparticles) value is strongly affected by the variables selected for the study. This is also reflected by the wide range of values for coefficients of the terms of Equation 3 and 4. The main effects of X_1 , X_2 , and X_3 represent the average result of changing 1 variable at a time from its low level to its high level. The interaction terms ($X_1 X_2$, $X_1 X_3$, $X_2 X_3$, X_1^2 , X_2^2 , and X_3^2) show how Y changes when 2 variables are simultaneously changed. The negative coefficients for all 3 independent variables indicate an unfavorable effect on Y, while the positive coefficients for the interactions between 2 variables ($X_1 X_2$ and X_3^2) indicate a favorable effect on Y. Among the 3 independent variables, the lowest coefficient value is for $X_2 X_3$ in equation 3 and 4, indicating that this variable is insignificant in prediction of Y.

The relationship between the dependent and independent variables was further elucidated by constructing contour plots.

Figure 4 A shows the Response surface plot and contour plot of silver nanoparticle synthesis from *Piper betle* extract and (i) shows the effect of Silver nitrate (mM) and plant extract on Silver Nanoparticle synthesis. It was obvious that when silver nitrate was at a low level, the effect of plant extract was negligible. When the silver nitrate concentration was at a higher level, silver nanoparticle synthesis steadily increased with increasing Y as 55 $\mu\text{g/mL}$. (ii) shows the effect of Sunlight exposure and Silver Nitrate on Silver Nanoparticle synthesis on increasing the silver nitrate concentration with increase in time of sunlight exposure with Y as 50 $\mu\text{g/mL}$ (iii) shows the effect of Sunlight exposure and Plant extract on Silver Nanoparticle Synthesis. The yield of silver nanoparticles steadily increased with the increase in time of sunlight exposure and silver nitrate concentration showing Y as 48 $\mu\text{g/mL}$.

Figure 4 B shows the Response surface plot and contour plot of silver nanoparticle synthesis from *Jatropha* extract *Piper betle* extract and (i) shows the effect of Silver nitrate (mM) and plant extract on Silver Nanoparticle synthesis.. It was obvious that when silver nitrate was at a low level, the effect of plant extract was negligible. When the silver nitrate concentration was at a higher level, silver nanoparticle synthesis steadily increased with increasing Y as 56 $\mu\text{g/mL}$. (ii) shows the effect of Sunlight exposure and Silver Nitrate on Silver Nanoparticle synthesis on increasing the silver nitrate concentration with increase in time of sunlight exposure with Y as 48 $\mu\text{g/mL}$ (iii) shows the effect of Sunlight exposure and Plant extract on Silver Nanoparticle Synthesis. The yield of silver nanoparticles steadily increased with the increase in time of sunlight exposure and silver nitrate concentration showing Y as 54 $\mu\text{g/mL}$.

The 3D response surface plots clearly show the complex interaction of the silver nitrate concentration, plant extract and the sunlight influence on the synthesise of silver nanoparticle. The plots indicate the linear relationship with incremental increase in yield of synthesis of silver nanoparticle. Like 3D response surface plots, 2D contour plots describe the analogues interpretation on relationship between the factors. Thus indicating the importance of the three parameters i) the silver nitrate, the donor of the silver ions for the synthesis [36], ii) the plant extract which is the biological reducing agent due to the presence of the nitrate reductase in synthesizing the silver nanoparticles [37] and iii) sunlight which act as a photo catalyst in the synthesis of silver nanoparticle [38,39]. This method of synthesis of silver nanoparticles through a green approach proves to be much effective and cheap method as it requires the natural plant extract and sunlight as a catalyst.

The observed and the predicted yield of silver nanoparticle synthesis is shown in Figure 5 from a) *Piper betle* and b) *Jatropha curcas* plant extract versus the predicted yield of silver nanoparticles and found that the BBD predictions are more accurate and near to the regression line suggesting the superiority of BBD in predicting silver nanoparticle synthesis. The clustering and concordance of points around the diagonal line confirms the compatibility of the model to predict the experiments.

Characterization of Silver nanoparticles by SEM:

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 (Figure 6). Thus through optimization of the critical factors stable uniform sized and shaped particles were generated.

Conclusion

In the present study, stable silver nanoparticles were green synthesized via biological reduction method with the help of *Piper betle* and *Jatropha curcas* extract. The major factors affecting the synthesis of nanoparticles were optimized using statistical tools. Statistical optimization of factors affecting the synthesis of silver nanoparticles using Plackett-Burman and Box-Behnken design appears to be a valuable tool for the synthesis of silver nanoparticle from *Piper betle* and *Jatropha curcas*. This study on varying nine different parameters on the synthesis of silver nanoparticle by Plackett Burman shows the optimum parameters to be chosen while synthesizing the silver nanoparticle. It is very clear from the statistical model that the parameters like plant extract, silver nitrate concentration and sunlight has greater influence on the synthesis of the silver nanoparticle. This was further analyzed by Box Behnken design. The plant extract serve as a source of reducing agent, silver nitrate solution serve as a donor for silver ions and sunlight acts as a catalyst enhancing the activation energy of the molecules and thereby giving more yield for the nanoparticle synthesis. The yield of silver nanoparticles can be increased by choosing the proper parameters and maintaining the optimal conditions. The BBD shows an obvious interaction between the parameters and hence proving the significance of the model. More stable and uniform sized nanoparticle were synthesized by optimizing the critical factors for the green synthesis of silver nanoparticle. Thus proving the green synthesis of silver nanoparticle is a cost effective, economic and stable method of synthesis.

Materials And Methods

Green synthesis of Silver nanoparticle:

The synthesis of silver nanoparticles was carried out from two leaf sources, *Piper betle* and *Jatropha curcas* under the optimized conditions. The leaves were washed thoroughly and air dried to remove the moisture. Phytochemical analysis were carried out to find the components in the leaf extract. 10 gm of the dried leaves were then crushed in mortar and pestle by adding adequate water making a solution of 100ml. This solution was then filtered using Whatman No.1 filter paper. The synthesized nanoparticles were collected by centrifugation and washed twice with distilled water to remove any contaminants.

Optimization of Silver nanoparticle:

The optimization of variables for the synthesis of silver nanoparticles was carried out by Plackett Burman design. Nine factors (pH, temperature, pressure, time of reaction, microwave radiation, UV radiation, concentration of plant extract, silver nitrate and sunlight) were used for analysis. All factors were tested in triplicates (S-1 to S-24). The maximum and minimum limits of experimental factors were determined in preliminary experiments. Aqueous solution of silver nitrate (AgNO_3) was prepared in distilled water. The leaf extract of *Piper betel* and *Jatropha curcas* was added to AgNO_3 solution and stirred continuously. Color change of the leaf extract was observed and optical density was noted at 430 nm using UV-Visible Spectrophotometer. After stabilization of reaction, a pellet was prepared by taking 20 ml of silver nanoparticles solution in falcon tubes and centrifuged at 10000 rpm for 15 min. Pellet was thoroughly purified by washing repeatedly with distilled H_2O and centrifuging for 10 min at 10000 rpm. Pellet was air dried for about 24 hrs. The yield for all the samples were calculated from the standard graph using the standard equation:

$y = 0.33x + 0.068$, where y is the optical density and

x is the concentration of silver nanoparticle.

Plackett Burman design:

PBD was applied to detect the best three significant variables and thereby improve the sequential optimization of process variables (8). PBD is much used for screening experiments, finding out the much impacted factors and heavily confounded with 2 factor interactions. It is a two factorial design and can identify the critical parameters required for elevated synthesis of silver nanoparticles by screening "n" variables through n+1 experiments. The experimental design screening of the variables is presented in Table 1.

PBD assumes that each parameter is independent and can be described by the first-order model:

$$Y = \beta_0 + \sum \beta_i X_i \quad (1)$$

where Y is the predicted target response (extraction yields of silver nanoparticles), β_0 : the model intercept, β_i : the regression coefficient, X_i : an independent parameter.

All the variables selected for the study were denoted as numerical factors and investigated at two level intervals designated as -1 for low level and +1 for high level. A nine factor Plackett Burman design was used to study the effect of variables on synthesis of silver nanoparticles. Variables chosen were pH X_1 (2, 10),

temperature, X_2 (20°C, 100°C), Pressure X_3 (5psi, 15psi), Time of reaction X_4 (2mins, 90mins), , Microwave radiation X_5 (10secs, 40sec), UV radiation X_6 (10mins, 40mins), concentration of plant extract, X_7 (0.1 g/ml, 0.4 g/ml), silver nitrate X_8 (0.001 M, 0.01 M) and sunlight X_9 (10mins, 90mins). X_{10} and X_{11} are considered as dummy variables. The values represented are lower (-) and upper limit (+) of each variable. The maximum and minimum limits for analysis were decided after performing a set of 14 preliminary experiments. Analysis was done using Statistica software which generated a 12 run table for 9 factors. All experiments were performed in triplicates to minimize experimental errors. Dummy variables (X_{10} and X_{11}) are used to estimate experimental errors in data analysis. Triplicates of all experiments were done and mean of AgNps biosynthesized was taken as the response. Based on the biosynthesized AgNps, the factorial experiment was analyzed using regression analysis and ANOVA.

Box Behnken Design:

The Box-Behnken design is a response surface methodology (RSM) design that requires only three levels (low, middle and high) to run an experiment. BBD was used to further optimize the synthesize conditions and was based on the parameters that were already screened as being significant factors by PBD. Plant extract (X_1), concentration of silver nitrate (X_2), and sunlight exposure (X_3) were selected for further optimization, with fluctuation ranges of 0.1-0.4 g/mL, 0.1 -1 mM and 30-90 min respectively. Each experiment was performed in triplicate. Analysis of variance (ANOVA) was used to analyze the experimental data from 17 experiments and the results were fitted by the response surface regression:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_{ij} \quad (2)$$

where Y is the predicted response, $X_i X_j$ represents coded independent variables, β_0 is the offset term, β_i is the i^{th} linear coefficient, β_{ii} is the i^{th} quadratic coefficient, and β_{ij} is the ij^{th} interaction coefficient. Statistica software was employed for experimental design as well as for regression and graphical analysis of the experimental data.

Characterization of Silver nanoparticle by SEM analysis:

After finding out the optimized parameters having major effect on the synthesis of nanoparticle, silver nanoparticles were synthesized at those optimized conditions. The size and shape of the nanoparticles synthesized were studied using SEM analysis. The characterization of the silver nanoparticles was carried out by Carl Zeiss ultra 55 SEM.

Declarations

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Conflict of Interest:

The authors have no conflict of interest to declare

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Not Applicable

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The authors have given consent to publish this article.

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Authors' contributions

Author Halima carried out the experimental work planning and execution. The optimization parameters selections, review of literature, calculation of the impact of the parameter s were decided. Author Dr. Archana contributed in editing, planning and overall guidance . Author Dr. Sivakiran contributed in modeling using software Statistica 7. All the authors read and approved the final manuscript.

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Tables

Table 1: Experimental variables (independent) at two levels (low and high) affecting the synthesis of AgNPs

S. N	Parameters	High Yield (+1)	Low Yield (-1)
1.	pH (X ₁)	10	2
1.	Temperature (X ₂) (degree Celsius)	100	20
1.	Pressure (X ₃) (psi)	15	5
1.	Time (X ₄) (mins)	2	90
1.	Microwave Radiation (X ₅) (secs)	40	10
1.	UV Radiation (X ₆) exposure (mins)	20	10
1.	Concentration of plant extract (X ₇) (g/ml)	0.1	0.4
1.	Silver Nitrate (X ₈) (mM)	1	0.25
1.	Sun Light (X ₉) (mins)	90	10

Table 2: Plackett Burman design for evaluation of 9 independent variables ($X_1 - X_9$) for synthesis of silver nanoparticles from a) *Jatropha curcas* extract b) *Piper betle* extract

Design: 11 Factor Screening Design (Spreadsheet1)												<i>Piper betle</i>	<i>Jatropha curcas</i>
Run	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	Yield (µg/mL)	Yield (µg/mL)
1	1	-1	1	-1	-1	-1	1	1	1	-1	1	41.51	41.53
2	1	1	-1	1	-1	-1	-1	1	1	1	-1	34.64	35.04
3	-1	1	1	-1	1	-1	-1	-1	1	1	1	29.2	29.11
4	1	-1	1	1	-1	1	-1	-1	-1	1	1	24.58	24.26
5	1	1	-1	1	1	-1	1	-1	-1	-1	1	33.56	33.58
6	1	1	1	-1	1	1	-1	1	-1	-1	-1	30.012	30.91
7	-1	1	1	1	-1	1	1	-1	1	-1	-1	33.75	33.31
8	-1	-1	1	1	1	-1	1	1	-1	1	-1	40.45	36.72
9	-1	-1	-1	1	1	1	-1	1	1	-1	1	31.38	30.97
10	1	-1	-1	-1	1	1	1	-1	1	1	-1	32.49	32.09
11	-1	1	-1	-1	-1	1	1	1	-1	1	1	31.04	30.57
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	20.46	19.57

Table 3: Regression coefficients, standard error, main effect, P-value for AgNPs synthesis

	<i>Piper Betle</i>						<i>Jatropha curcas</i>					
	Regressn	Std.Err.	t(2)	P	-95.%	+95.%	Regressn	Std.Err.	t(2)	P	-95.%	+95.%
Mean/Interc	26.3570	3.266337	8.06929	0.015013	12.3031	40.41093	27.3550	0.840485	32.5467	0.000064	24.6802	
1	0.3959	0.171321	2.31093	0.147043	-0.3412	1.13305	0.2447	0.042753	5.7229	0.01110588	0.1086	
2	0.0115	0.017132	0.67323	0.570171	-0.0622	0.08525	0.0031	0.004127	0.7608	0.502113	-0.0100	
3	0.2644	0.137057	1.92909	0.193504	-0.3253	0.85410	0.2861	0.034202	8.3641	0.003584	0.1772	
4	0.0000	0.000099	0.19435	0.863853	-0.0004	0.00044	0.0256	0.004312	5.9396	0.009541	0.0119	
5	0.0403	0.045686	0.88239	0.470644	-0.1563	0.23688	0.0627	0.011004	5.6984	0.010716	0.0277	
6	-0.2551	0.136975	-1.86207	0.203640	-0.8444	0.33430	-0.2782	0.037944	-7.3327	0.005240	-0.3990	
7	-22.1021	4.568563	-4.83786	0.040169	-41.7590	-2.44513	-24.3112	1.140072	-21.3243	0.000226	-27.9394	
8	7.9253	1.827425	4.33685	0.049272	0.0625	15.78805	8.0498	0.456029	17.6520	0.000396	6.5985	
9	0.0589	0.017132	3.43941	0.075131	-0.0148	0.13264	0.0502	0.004275	11.7440	0.001327	0.0366	

Table 4: Sum of Squares(SS), Degree of Freedom (Df), Mean square(MS), F-value (F), P-Value(p)

Plant extract	<i>Jatropha curcas</i>					<i>Piper betle</i>				
Parameters	SS	Df	MS	F	P	SS	Df	MS	F	P
X ₁	10.6095	1	10.6095	32.7515	0.010588	22.5263	1	22.52632	5.34039	0.147043
X ₂	0.1875	1	0.1875	0.5788	0.502113	1.9118	1	1.91183	0.45324	0.570171
X ₃	22.6621	1	22.6621	69.9574	0.003584	15.6972	1	15.69723	3.72140	0.193504
X ₄	11.4282	1	11.4282	35.2785	0.009541	0.1593	1	0.15933	0.03777	0.863853
X ₅	10.5188	1	10.5188	32.4714	0.010716	3.2843	1	3.28428	0.77862	0.470644
X ₆	17.4176	1	17.4176	53.7679	0.005240	14.6255	1	14.62548	3.46731	0.203640
X ₇	147.3036	1	147.3036	454.7237	0.000226	98.7242	1	98.72420	23.40489	0.040169
X ₈	100.9374	1	100.9374	311.5919	0.000396	79.3352	1	79.33524	18.80828	0.049272
X ₉	44.6781	1	44.6781	137.9205	0.001327	49.8983	1	49.89832	11.82957	0.075131
Error	0.9718	3	0.3239			8.4362	2	4.21810		
Total SS	522.4125	12				350.2704	11			

Table 5: Observed and predicted values on yield of nanoparticles

	<i>Piper betle</i>			<i>Jatropha curcas</i>		
	Observed	Predicted	Resids	Observed	Predicted	Resids
1	41.53000	41.53987	-0.00987	41.51000	41.66035	-0.150352
2	35.04000	35.03342	0.00658	34.64000	34.53977	0.100235
3	29.11000	29.77382	-0.66382	29.20000	29.03320	0.166802
4	24.26000	23.54618	0.71382	24.58000	24.31296	0.267037
5	33.58000	32.21551	1.36449	33.56000	33.66023	-0.100235
6	30.91000	31.62053	-0.71053	30.01200	30.22892	-0.216920
7	33.31000	32.64618	0.66382	33.75000	33.91680	-0.166802
8	36.72000	36.71342	0.00658	40.45000	40.34977	0.100235
9	30.97000	29.60222	1.36778	31.38000	31.43012	-0.050117
10	32.09000	33.45449	-1.36449	32.49000	32.38977	0.100235
11	30.57000	31.23053	-0.66053	31.04000	30.82308	0.216920
12	19.57000	20.28382	-0.71382	20.46000	20.02352	0.436481

Table 6: Box–Behnken experimental design and yield of silver nanoparticle

Standard Run	Plant Extract (g/mL)	AgNO ₃ mM	Sunlight mins	Experimental (Piper betle) Conc. (µg/mL)	Experimental (Jatropha curcas) Conc. (µg/mL)
1.	0.100000	0.10000	60.00000	28.6	48.89
2.	0.400000	0.10000	60.00000	35.68	40.71
3.	0.100000	1.000000	60.00000	50.38	51.78
4.	0.400000	1.000000	60.00000	40.8	43.6
5.	0.100000	0.5000	30.00000	47.29	47.7
6.	0.400000	0.5000	30.00000	37.71	39.55
7.	0.100000	0.5000	90.00000	48.22	50.51
8.	0.400000	0.5000	90.00000	38.64	42.34
9.	0.250000	0.10000	30.00000	38.26	39.86
10.	0.250000	1.000000	30.00000	43.37	42.75
11.	0.250000	0.10000	90.00000	39.19	42.66
12.	0.250000	1.000000	90.00000	44.30	45.56
13.	0.250000	0.5000	60.00000	41.29	43.50
14.	0.250000	0.5000	60.00000	41.29	43.50
15.	0.250000	0.5000	60.00000	41.29	43.50

Table 7: Analysis of variance for the influence of the selected variables on silver nanoparticle synthesis

Source of plant	Source of variation	SS	Df	MS	F	p
Piper betel	(1)Plant Extract (g/mL) L+Q	68.5189	2	34.25943	2.214639	0.204752
	(2)Silver Nitrate (mM) L+Q	187.8295	2	93.91475	6.070949	0.045949
	(3)Sunlight Exposure (Min) L+Q	17.1606	2	8.58029	0.554657	0.605960
	1*2	61.5135	1	61.51347	3.976427	0.102711
	1*3	0.0000	1	0.00000	0.000000	1.000000
	2*3	0.0000	1	0.00000	0.000000	1.000000
	Error	77.3477	5	15.46954		
	Total SS	405.6015	14			
Jatropha curcas	(1)Plant Extract (g/mL) L+Q	156.1257	2	78.06283	567518.7	0.000000
	(2)Silver Nitrate (mM) L+Q	16.8839	2	8.44195	61373.2	0.000000
	(3)Sunlight Exposure (Min) L+Q	19.4413	2	9.72064	70669.3	0.000000
	1*2	0.0000	1	0.00000	0.0	0.928422
	1*3	0.0001	1	0.00010	0.7	0.432791
	2*3	0.0000	1	0.00003	0.2	0.672477
	Error	0.0007	5	0.00014		
	Total SS	194.8668	14			

Figures

Figure 1

a) plant extract b) reduction to AgNp

Figure 2

Pareto chart for standardized Effects a) Piper betle b) Jatropha curcas

Figure 3

Half-normal probability plot of effects for choosing the statistically significant effects in Plackett–Burman screening design.

Figure 4

Response surface plot and contour plot of A) Piper betle and B) Jatropha curcas the combined effects of (i) Silver Nitrate and Plant extract (ii) Sunlight exposure and Silver Nitrate (iii) Sunlight exposure and Plant extract on Silver Nanoparticle Synthesis.

Figure 5

Observed and predicted value of yield of silver nanoparticle from a) Piper betle and b) Jatropha curcas plant extract (green synthesis)

Figure 6

SEM image of silvernanoparticles from a) *Jatropha curcas* and b) *Piper betle* leaf extract