PARAMETRIC OPTIMIZATION OF SYNTHESIS OF SILVER NANOPARTICLES FROM *Mangifera indica* AND *Prunus dulcis* EXTRACTS AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract. Optimization of process parameters is crucial to the deployment of nanotechnology as a competitive source of novel materials to many fields. The present study has lent credence to the simplicity, environmental friendly, cost effective and quick potentials of using plant extracts as source of capping and stabilization agents in the synthesis of silver nanoparticles (AgNPs). Aqueous leaf extracts of *Mangifera indica* and *Prunus dulcis* were used in synthesis of silver nanoparticles. The study was designed with Box Behnken Design (Minitab® 17) to optimize temperature (25 – 35°C), pH (6 – 8) and time of reaction (6 – 24hours). Fifteen runs were obtained for each sample which determined the value of each parameter used for the synthesis. Results obtained were subjected to Response Optimizer (Minitab® 17) which predicted optimum conditions for synthesis of silver nanoparticles as 25°C at pH 8 and 10.24 hours with predicted maximum yield of 2.53 for *Prunus dulcis*. However, the actual yield of silver nanoparticles under these conditions was 2.64. For *Mangifera indica* leaf extract, the predicted optimum conditions were 31.4°C at pH of 8.0 and 9.39 hours with predicted maximum yield of 2.55. Nevertheless, the actual yield under the optimum conditions was 2.61. Results show that *Prunus dulcis* extract has relatively higher potential yield for silver nanoparticles than *Mangifera indica* extracts. UV-Vis spectrophotometer showed that the absorbance for synthesized silver nanoparticles using both plant extracts peaked between 400 – 430nm. Silver nanoparticles from both plants showed activity against *Bacillus subtilis* and *Pseudomonas aeruginosa,* though *B. subtilis* was more sensitive. However, silver nanoparticle from *Prunus dulcis* was less effective against the bacteria.

Keywords: nanotechnology; green synthesis; leaf extracts; parameters.

INTRODUCTION

Since the last few decades, nanotechnology has been witnessing unprecedented attention by many researchers. This is due to the rapidly increasing global problems associated with environmental pollution which has paved way for "green" eco-friendly technologies and chemicals are becoming increasingly popular [9, 10, 16]. Nanoparticles (NPs) have drawn more attention due to modification of properties arising from size effects, catalytic, electronic and optical properties of the monometallic nanoparticles [7]. Consequently, researcher are exploring the use of biological systems for the synthesis of biocompatible metal and semiconductor nanoparticles [13] in order to replace synthetic chemicals which are associated with toxicity and threat to human life and environmental sustainability [5, 15].

Thus, there is an increasing demand for green nanotechnology [8]. There are many reports of successful extracellular and intracellular synthesis of different types of nanoparticles using bacteria, fungi and plants extracts [19]. In contrast to other biological agents, plant extract – dependent synthesis of nanoparticles has been widely accepted because it is simple, cost effective, less time consuming and eco-friendly. Also, wider distribution of metabolites, more availability of plants and safer handling are other competitive advantages of plant extract – based synthesis of nanoparticles [19]. This approach is made possible by different types of phytochemicals and active metabolites found in plant extracts such as terpenoids, gums, fats, enzymes, flavonoids, ketones, aldehydes, amides and carbohydrates, carboxylic acids etc [3]. These phytochemicals are readily obtainable in different combinations and concentrations in leaf, bark and root extracts of virtually all plants.

The size and shape of NPs affect their antimicrobial activity, with smaller particles exhibiting higher activity. Several mechanisms have been proposed for antibacterial activity of silver nanoparticles (AgNPs) which include release of silver ions into the cells [1, 25], attachment and disturbance of cell wall permeability and impairment of cellular respiration. Also, NPs may penetrate the cell, interact with phosphorus and sulphur-containing vital compounds which include DNA and protein and consequently disrupt their activities.

However, the yield and properties of resulting metal nanoparticles synthesized using plant extract based approach are dependent on temperature, reaction time and pH of the medium as well as the concentration and type of extract used. The range of variation in size of nanoparticles with temperature is about 5-300nm with smaller and more spherical particles synthesized at higher temperature of about 65°C [28] but with reduced amount [24]. Similarly, varying the pH of the reaction medium between 3 and 9 has yielded different shapes like triangle, hexagons, spheres, and rods [9].

In order to improve on the yield and properties of synthesized silver nanoparticles, the interaction effects of time reaction, temperature and pH of reacting medium was undertaking using Box Behken design. The parameters were optimized and used to synthesize

silver nanoparticles which were characterized and their antibacterial activity determined.

MATERIAL AND METHODS

Collection of plant samples and preparation of aqueous extracts

Leaf samples of *Mangifera indica* and *Prunus dulcis* were collected from the Botanical garden of the Federal University of Technology, Owerri, Nigeria and identified by a plant taxonomist in the same University. Each of the leaf samples was dusted, washed under running tap water and completely dried under the sun. The dried samples were then pulverized to fine powder and preserved in sterile containers until used.

The preparation of aqueous leaf extracts of each plant was done by weighing 20g of the pulverized sample into a conical flask containing 100ml distilled water. The mixture was placed on hot plate and allowed to decoct for 20 minutes. Then the content was filtered with Whatman No 1 filter paper and the filtrate stored until used.

Design of study for optimization

Box-Behnken design (Minitab® 17) was used to design the optimization of parameters including temperature (varied between 25 and 35°C), pH (varied between 6 and 8) and time of reaction (varied between 6 and 24 hours). The design resulted in 15 runs each characterized with different mix of the three parameters as indicated in table 1.

Table 1. Box-Behnken design for optimization study

Run order	Temperature (°C)	pH	Time (ours)
1	25	6	15
2	35	6	15
3	25	8	15
4	35	8	15
5	25	7	6
6	35	7	6
7	25	7	24
8	35	7	24
9	30	6	6
10	30	8	6
11	30	6	24
12	30	8	24
13	30	7	15
14	30	7	15
15	30	7	15

Synthesis and characterization of AgNPs

Plant extract based silver nanoparticles (AgNPs) were synthesized by adding 20ml of 10mM silver nitrate ($AgNO_3$) solution to 30 conical flasks grouped into two of 15 flasks each. One group of the flasks contained 10ml of *Mangifera indica* extract while the other 10ml of *Prunus dulcis* extract. For each flask, the pH and temperature were maintained using phosphate buffer and hot plate respectively. The flasks were then sealed and synthesis of silver nanoparticles was followed by observing change in colour of the solutions. In line with the time of reaction defined by the Box Behken design, the absorbance (yield) of nanoparticles synthesized in each flask was determined using Ultraviolet–visible Spectrophotometer (Labman) at 200 - 600nm wavelength with a resolution of 1.

Similarly, the optimum pH, temperature and time of reaction for synthesis of silver nanoparticles were predicted with Response Optimizer (Minitab® 17). These optimum values were then applied to synthesize silver nanoparticles using each leaf sample. They were characterized and their antibacterial study conducted.

Antibacterial assay

The antibacterial analysis was conducted using clinical isolates of *Pseudomonas aeruginosa* and *Bacillus subtilis* collected from Anthony Van Leuwenhoek's Research Center, Owerri, Imo State, Nigeria. The isolates were identified following cultural and biochemical tests as described by [4, 6]. The time kill kinetics approach was adopted for studying the antibacterial activity of AgNPs synthesized using each plant extract under optimum conditions. Eight sterilized test tubes were grouped into 2, each for a given extract based silver nanoparticles. The first two test tubes in each group were designated *Bacillus subtilis* "N" and "D", while the other 2 tubes were designated *Pseudomonas* "N" and "D". Nutrient broth (1 ml) was put into all of the test tubes followed by addition of 1ml *Mangifera indica* and *Prunus dulcis* extracts based silver nanoparticles to tubes labeled "N" in groups 1 and 2 respectively. Then 1ml of sterilized distilled water was added to the tubes labeled "D" in each group. Overnight broth culture (1ml) of *Bacillus subtilis* and *Pseudomonas aeruginosa* each was inoculated in appropriate tubes as labeled. The tubes were incubated at 37 °C and bacterial growth was monitored by determining the absorbance of each medium at time intervals of 2 hours until 18 hours using UV- vis spectrophotometer at wavelength 600nm.

RESULTS

Synthesis of silver nanoparticles

The results obtained are indicative of the suitability of *Mangifera indica* and *Prunus dulcis* leaves for synthesis of silver nanoparticles. For each of the experimental set up, after addition of $AgNO_3$ to the flask containing the plant extract, the colour of the solution was observed to change from brown to a deeper brown. However, the rate of change of colour differed among the set ups. According to the time of reaction defined in the Box Behken design, the absorbance of each mixture was read and the results obtained for each plant extract based silver nanoparticles are shown in figure 1.

On characterization, the absorbance spectra for *Mangifera indica* and *Prunus dulcis* leaf extracts – based silver nanoparticles synthesized under optimum conditions were observed to peak between 400 – 430nm wavelength as shown in figure 2. The spectra also indicated a broader absorbance peak for

nanoparticles synthesized with *Prunus dulcis* extract than *Mangifera indica* extract. This makes room for synthesis of more nanoparticles.

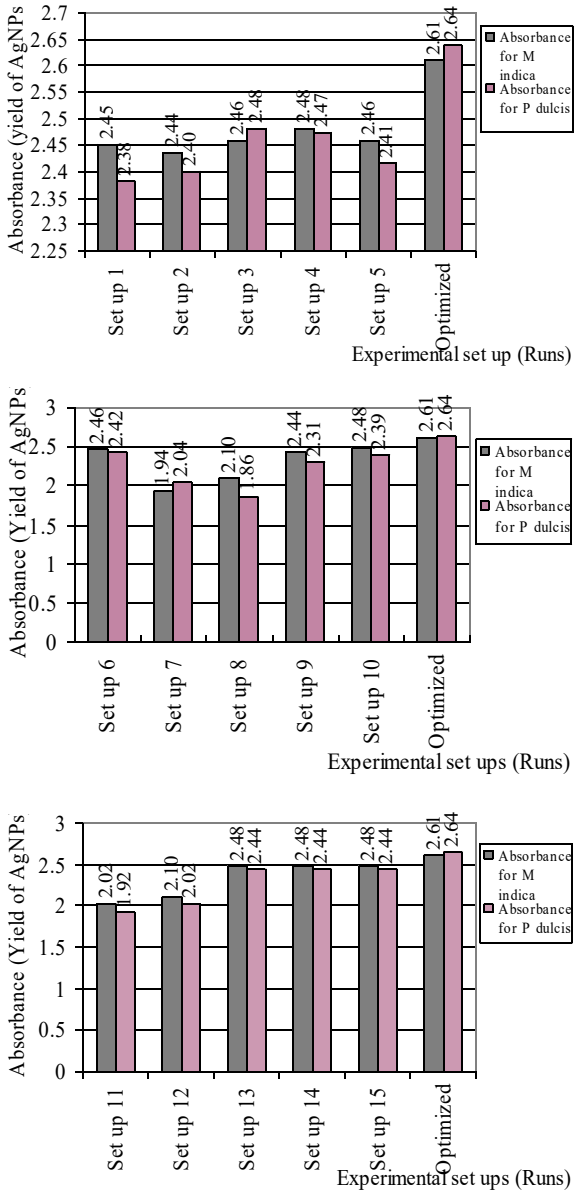

Figure 1. Absorbance of silver nanoparticles in study with different extracts

Optimization of temperature, pH and time of reaction

The results obtained from the optimization of temperature, pH and time of reaction using Response Optimizer (Minitab® 17) predicted that the optimum conditions were 25°C at pH 8 and 10.24 hours with predicted maximum yield of 2.53 for *Prunus dulcis*. The yield of silver nanoparticles synthesized under these conditions was 2.64. The predicted optimum conditions using *Mangifera indica* leaf extract were 31.4°C at pH of 8.0 and 9.39 hours with predicted maximum yield of 2.55. However, the actual yield under the optimum conditions was 2.61. The results show that *Prunus dulcis* extract has relatively higher potential yield for silver nanoparticles than *Mangifera indica* extracts. Moreover, there is interplay between time of reaction and temperature of the medium. While *P. dulcis* achieved maximum yield at reduced temperature, it required more time unlike *Mangifera indica* that achieved it at reduced time but higher temperature.

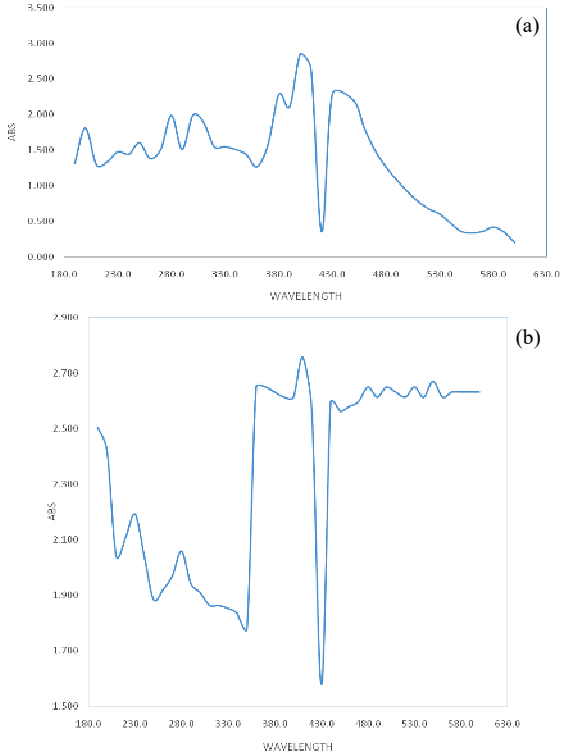

Figure 2. Absorption spectra for (a) *Mangifera indica* and (b) *Prunus dulcis* leaf extracts – based silver nanoparticles

Antibacterial screening

The AgNPs synthesized with the extracts demonstrated marked antibacterial activity against both isolates. However, *Bacillus subtilis* was more sensitive to the plant extracts based silver nanoparticles than *P. aeruginosa*. This is depicted by the sharp decline in its absorbance as shown in figures 3 and 4.

Moreover, a comparison of the antibacterial activities of the plant extract based silver nanoparticles indicated that *Prunus dulcis* leaf extract based silver nanoparticles exhibited lesser antibacterial activity than *Mangifera indica* leaf extract based silver nanoparticles. This is observable from the time it took each extract to impact a decline in the population of *B. subtilis* and *P. aeruginosa.* While decline in populations of *B. subtilis* and *P. aeruginosa* was observed after 4 hours of incubation in *M. indica* leaf extract based silver nanoparticles, it took 10 hours of incubation in *P. dulcis* leaf extract based silver nanoparticles before decline could be observed as shown in figures 5 and 6.

DISCUSSION

Besides *Mangifera indica* and *Prunus dulcis* which were used in this study, silver nanoparticles have also been synthesized using many different plant extracts

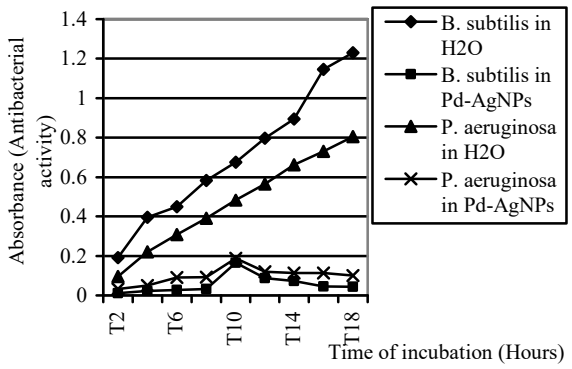

Figure 3. Time kill kinetics of antibacterial of *Prunus dulcis* (Pd) leaf extract – based silver nanoparticles (AgNPs)

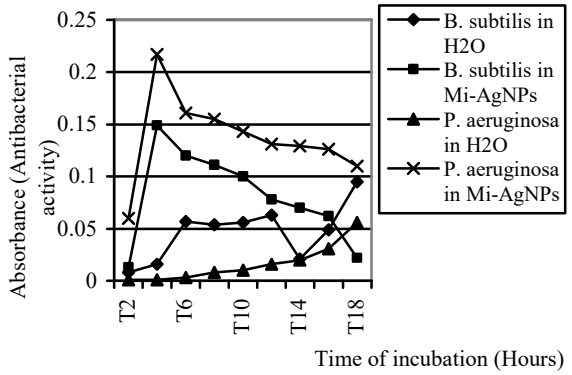

Figure 4. Time kill kinetics of antibacterial of *Mangifera indica* (Mi) leaf extract – based silver nanoparticles (AgNPs)

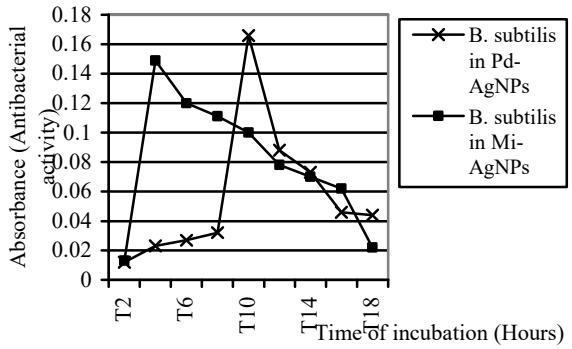

Figure 5. Comparison of antibacterial activities of *Prunus dulcis* (Pd) and *Mangifera indica* (Mi) leaf extracts – based silver nanoparticles (AgNPs) against *Bacillus subtilis*

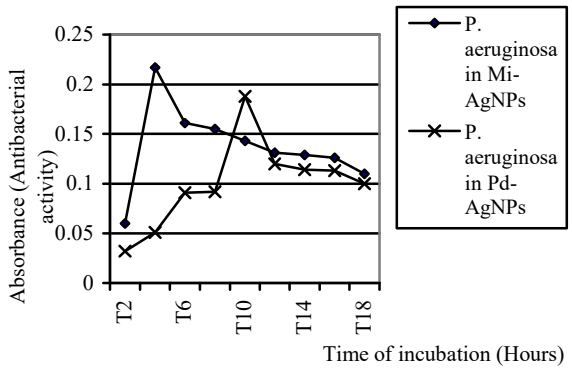

Figure 6. Comparison of antibacterial activities of *Prunus dulcis* (Pd) and *Mangifera indica* (Mi) leaf extracts – based silver nanoparticles (AgNPs) against *Pseudomonas aeruginosa*

including leaf extract of *Azadirachta indica* [18], *Cynanchum viminale* and *Cynanchum sarcomedium* [12], *Artemisia nilagirica* [30], leaf extract of *Volkameria inermis* [14]. After addition of silver nitrate to the plant extract, the colour change from brown to deep brown was observed in this study. Other researchers have equally reported similar finding. Mathur [18] reported colour change from yellow to brown. Kannan and Ernest [12] observed colour change to yellowish brown/dark brown colored while it was from yellow to intense brown [14].

Also, the absorbance recorded in this study peaked around 400 – 430 nm. The characteristic peak of absorbance for silver nanoparticles was reportedly observed from 400 to 450 nm using UV-Vis analysis [23]. Mathur [18] have reported peak absorbance at 400 nm while [14] observed a peak at 430 nm. Similarly, the peak of absorbance was at 500 nm with value of 3.21 and at 400 nm with value of 1.87 for *Cynanchum viminale* and *Cynanchum sarcomedium* extracts – based silver nanoparticles [12].

It has been reported that spherical nanoparticles have characteristic absorption peak in the range of 400-420 nm. Polydispersion of nanoparticles have been implicated in the broadening of absorption peak of nanoparticles [20, 26]. However, narrow λmax is better peak for AgNPs [17]. It is reported that the concentration of substrate, bio-catalyst, the temperature, pH, incubation time and light affect the size, morphology, and properties of resulting nanoparticles [29, 30].

In the optimization study, the Response Optimizer (Minitab ® 17) gave the optimum conditions as 25°C, pH 8 and 10.24 hours with predicted maximum yield of 2.53 for *Prunus dulcis*. However, the actual yield of silver nanoparticles following the conditions was 2.64. For *Mangifera indica* leaf extract, the optimum conditions obtained were 31.4°C, pH of 8.0 and 9.39 hours of reaction with predicted maximum yield of 2.55. The actual yield following the predicted optimum conditions was eventually 2.61 This is comparable to the optimal conditions reported as follows; time of reaction 10 minutes, at 80°C, with 1mM silver nitrate, pH of 8 and stoichiometry of 1mM silver nitrate to methanolic extract of 9:1 [12]. This further supports our earlier assertion that temperature and time have indirect relationship in their effects on the synthesis of silver nanoparticles. The higher the temperature, the lower the time required for synthesis.

Many researchers have reported antibacterial activity of plant extract based silver nanoparticles against different gram positive and gram negative bacteria including *Escherichia coli* [27], *Bacillus subtilis* and *Pseudomonas aeruginosa* [2], *Pseudomonas aeruginosa* and *E. coli and Klebsiella pneumonia* [31], *Escherichia coli* and *Staphylococcus aureus* [11], *Bacillus subtilis, Escherichia coli* and *Bacillus vallismortis* [21]. The antibacterial activity of silver nanoparticles has been attributed to their higher surface to volume ratio in comparison to their bulk

counterparts. This property is known to enhance the interactions of silver nanoparticles with the bacterial surfaces. For instance, the death of bacterial cells is initiated by the interaction between silver nanoparticles and sulphur and phosphorus-containing components of the cells which affect the respiratory chain and cell division [22].

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Received: 21 December 2019
Accepted: 16 February 2020
Published Online: 21 February 2020
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House