

Purification of simulated waste water using green synthesized silver nanoparticles of *Piliostigma thonningii* aqueous leave extract

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Purification of simulated waste water using green synthesized silver nanoparticles of *Piliostigma thonningii* aqueous leave extract

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Abstract

Synthesis of nanoparticles from various biological systems has been reported, but among all such systems, biosynthesis of nanoparticles from plants is considered the most suitable method. The use of plant material not only makes the process eco-friendly, but also the abundance makes it more economical. The aim of this study was to biologically synthesize silver nanoparticle using *Piliostigma thonningii* aqueous leaf extract and applied in the purification of laboratory stimulated waste with optimization using the different conditions of silver nanoparticle production such as time, temperature, pH, concentration of silver nitrate and volume of the aqueous extract. The biosynthesized silver nanoparticles were characterized by UV-visible spectrophotometry, nanosizer, energy dispersive x-ray analysis (EDX), transmission electron microscopy (TEM) and Fourier transform infrared (FTIR) spectroscopy. The time intervals for the reaction with aqueous silver nitrate solution shows an increase in the absorbance with time and became constant giving a maximum absorbance at 415 nm at 60 min of incubation. The pH of 6.5, temperature 65 °C, 1.25 mM of silver nitrate and 5 ml of plant extract was the best condition with maximum absorbance. The results from nanosizer, UV-vis and TEM suggested the biosynthesis silver nanoparticle to be spherical ranging from 50 nm to 114 nm. The EDX confirmed the elemental synthesis of silver at 2.60 keV and FTIR suggested the capping agent to be hydroxyl (OH) group with $-C=C$ stretching vibrations. The synthesized silver nanoparticle also shows heavy metal removal activity in laboratory simulated waste water. The safety toxicity studies show no significant difference between the orally administered silver nanoparticles treated water group and control group, while the histopathological studies show well preserved hepatic architecture for the orally administered silver nanoparticle treated waste water group when compared with the control group. Therefore, it can be concluded that the biosynthesized silver nanoparticles have efficient ability in heavy metal removal without sub chronic adverse effects in experimental rats.

Keywords: silver nanoparticles, heavy metals, contamination, waste water, removal, *Piliostigma thonningii*

Classification numbers: 2.03, 4.02

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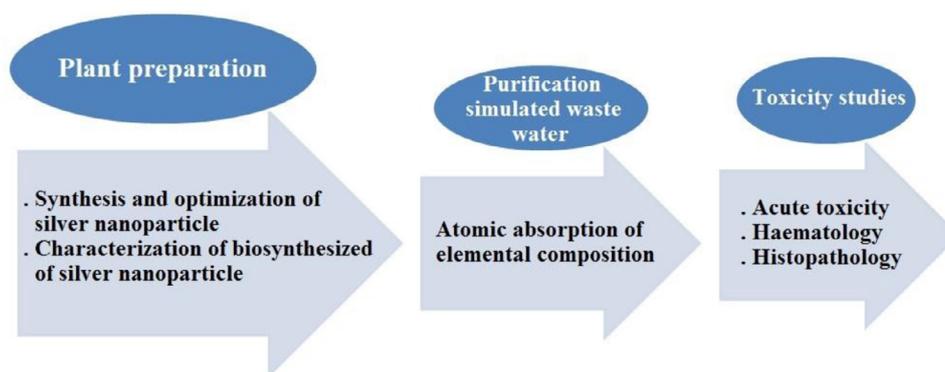


Figure 1. Flowchart for the overall process.

1. Introduction

Because of the rapid increase in world population and global warming, water resources are fast dwindling. Therefore, discrete utilization of water resources and reuse of treated wastewater for different purposes have been recognized as the most effective ways of conserving the limited resources of fresh-water [1]. The presence of microorganisms and heavy metals is the main indication of water contamination. Heavy metal contamination of water is a public health concern, with several health risks associated with it [2, 3].

Organisms such as *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Vibrio* spp. and *Cryptosporidium* are known to be transmitted by water and cause ill health in communities consuming water contaminated by bacteria [4].

Presently, metal nanoparticles especially gold and silver ones, are of particular interest to researchers due to their unique properties and wide application in medical field. Biosynthesis is a better alternative to physical and chemical methods [5, 6] due to eco-friendly, cost effective and less time required. Various materials are categorised as biologic for nanoparticle synthesis, these includes plants extract, bacteria, fungi enzymes and actinomycetes [7]. But plant extracts have been the major focus due to their abundant nature and the phytochemical composition [8, 9]. The plant *Piliostigma thonningii* is widely used in Africa for treatment of diseases such as ulcers, diarrhoea, dysentery, worms and other intestinal problems with activity based on the phytochemical constituents [10, 11].

Therefore, based on the reported phytochemical constituents of the plant, this study carried out to investigate the green synthesis of silver nanoparticles by aqueous leaves extract of *P. thonningii*. Also, using different factors like temperature, pH, time, silver nitrate (AgNO_3) concentration and leaf extract concentration to optimized synthesis of silver nanoparticles which can then be used in simulated waste water treatment. To confirm the safety use, the acute and sub chronic toxicity of the treated waste water was carried out on experimental rats. The overall process is shown in figure 1.

2. Materials and methods

2.1. Materials

Fresh leaves of *P. thonningii* were collected from military Barracks Minna, Niger State, Nigeria. Waste water was



Plate 1. Aqueous extract of *P. thonningii*.

simulated at the Centre for Biotechnology Research (STEP B), Federal University of Technology Minna, Niger State. Pure silver nitrate (AgNO_3) used in this work was obtained from Sigma Aldrich Company, USA. Experimental animals were obtained from the Department of Biochemistry, Federal University of Technology, Minna.

2.2. Methods

2.2.1. Plant extract preparation Fresh leaves of *P. thonningii* were washed with clean water, and then air dried for 15 d at room temperature to prevent the destruction of thermo labile constituent of the plant by direct sun rays. The leaves were destalked and milled into coarse powder. Twenty five (25 g) of the leaves powdered of *P. thonningii* was then weighed and put into 1000ml a conical flask containing 500ml distilled water, mixed and boiled for 25 min. The aqueous extract was filtered using a muslin cloth and then through a filter paper (Whatman no. 1). The filtrate (plate 1) was kept in a refrigerator for the biosynthesis of silver nanoparticles.

2.2.2. Qualitative phytochemical screening The qualitative phytochemical analysis was carried out on the plant extract according to the methods of Trease and Evans [12] and Sofowora [13].

2.2.3. Synthesis of silver nanoparticles A 5 ml of aqueous extract of *P. thonningii* was added to 95 ml of aqueous solution of 1 mM AgNO_3 and heated with stirrer at 70 °C for 60 min

Table 1. Central components investigational factors.

Factors	Concentration of AgNO ₃ (mM)	Concentration of leaf extract (ml)	Temperature (°C)	pH	Time (min)
SNP1	1.00	7.50	75	6.5	60
SNP2	1.00	7.50	65	7.0	60
SNP3	1.25	5.00	65	6.5	60
SNP4	1.13	9.22	70	7.5	55
SNP5	1.00	7.50	65	6.5	50

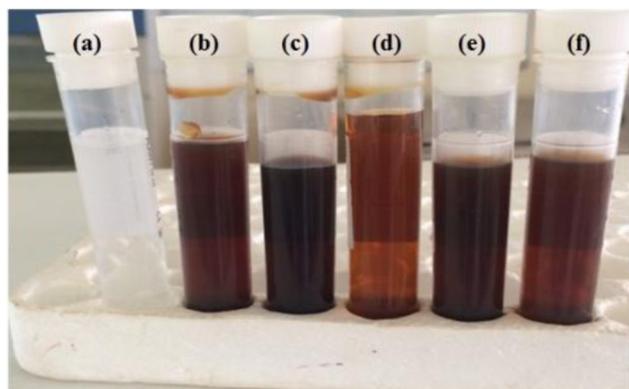


Plate 2. 1 mM silver nitrate (a), biosynthesized silver nanoparticles (b) SNP1, (c) SNP2, (d) SNP3, (e) SNP4 and (f) SNP5.

Table 2. Phytochemical constituent of *P. thonningii* aqueous extract.

Phytochemicals	Interference
Tannin	+
Flavonoids	+
Anthraquinones	+
Phenol	+
Steroids	+
Saponins	+
Phlobatannins	-
Alkaloids	+
Terpenoids	+
Glycosides	+

Keys: (+) present, (-) absent.

and pH 7 as described by Ahmad *et al* [14] to confirm the silver nanoparticle synthesis. In order to improve the synthesis method, an optimization was carried out using the central components Design of Experiments ‘one factor-at-a time’ (table 1) and the product of each reaction (plate 2) was characterized using UV-spectroscopy measurements (UV-1800 Shimadzu spectrophotometer) for various condition factors over a range of 300–700 nm.

2.2.4. Characterization of biosynthesized silver nanoparticles (SNP3) The hydrodynamic diameter of the selected condition factor (SNP3) nanoparticles was determined by dynamic light scattering (DLS) with the help of a Zetasizer 3000 (Malvern Instruments, UK) using an argon ion laser beam at a wavelength of 450 nm. The capping and reducing functional groups of SNP3 were evaluated using Fourier

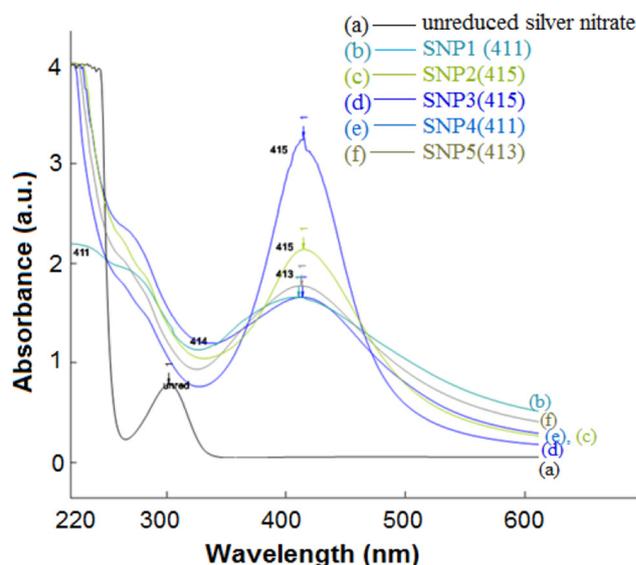


Figure 2. UV-vis spectra of silver nanoparticles synthesized by different condition factors.

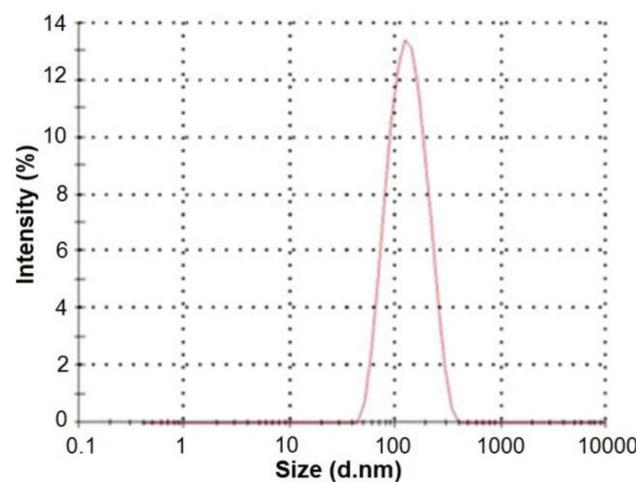


Figure 3. Particle size of the biosynthesized silver nanoparticle SNP3.

transform infrared (FTIR) spectroscopy. This was performed by using freeze-dried biosynthesized gold nanoparticles mounted on the copper stub. The morphology image was studied using scanning electron microscope (SEM) (Hitachi, model S-3400N) with secondary electron detectors at an operating voltage of 30 kV. Energy dispersive x-ray (EDX) spectroscopy of SNP3 was done on S-3400N, Hitachi instrument [14, 15].

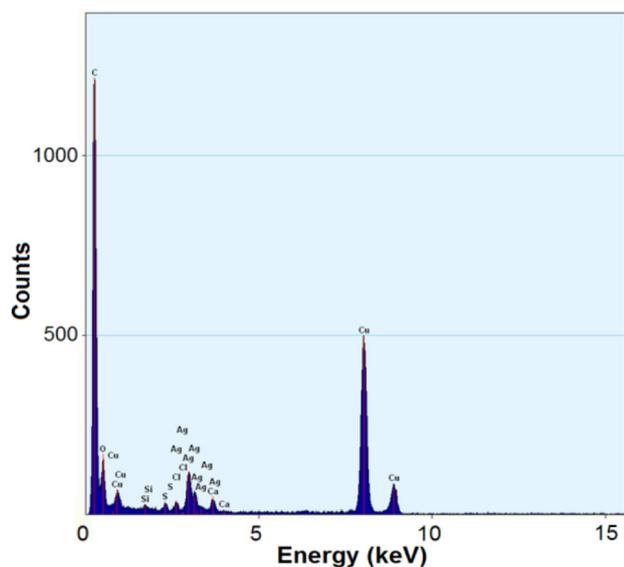


Figure 4. EDX spectrum of optimized biosynthesized silver nanoparticles SNP3.

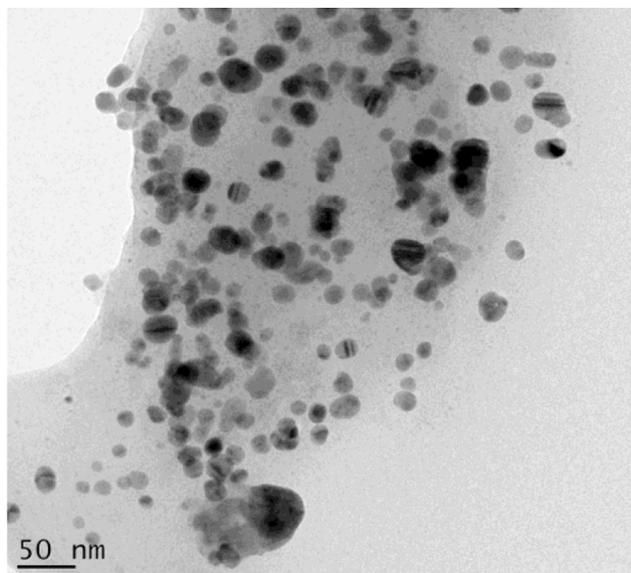


Figure 5. TEM image of bio-synthesized silver nanoparticle SNP3.

2.2.5. Adsorption process of laboratory simulated water by SNP3 Different concentrations of working and standard solution of copper ions, iron III sulphate, manganese and lead II sulphate of the stock solution of 1 g l^{-1} of the metals were prepared using pure lead sulphate, iron sulphate, manganese sulphate and copper sulphate and distilled water. Experiments were performed on three periods (20, 40, 60 min), three levels of pH (3, 7 and 9), two different concentrations of the metal ions (10, 20 mg l^{-1}), and absorbent dose (0.2 g per 100 ml). Then the metal ions solution contact with the adsorbent was shaken at specified time on 200 rpm orbital shaker.

2.2.6. Determination of acute toxicity (LD_{50}) The lethal dose LD_{50} of the biosynthesized silver nanoparticles (SNP3) was determined by oral administration to experimental rats according to Lorke method [16].

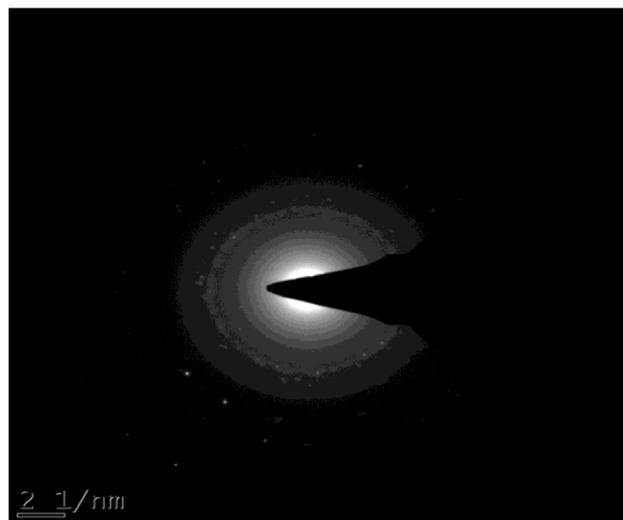


Figure 6. SAED image of bio-synthesized silver nanoparticle SNP3.

2.2.7. Determination of subchronic toxicity Ten rats (10) were divided into groups with five (5) rats per group. The normal control group received distilled water and the SNP3 group received oral 300 mg kg^{-1} body weight of nanoparticle treated water based on the LD_{50} for 28 d. On the 29th day, rats were fasted overnight and anesthetized with ether and sacrificed. The blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes for haematological evaluation and the liver were preserved in 70% formalin for histopathology studies.

The preparation of tissue sections for histological examination under light microscope followed the standard embedding and H-E staining protocol. The photomicrographs were captured at $100\times$ using the software Presto Image Folio package.

2.2.8. Statistical analysis Values are represented as mean \pm standard error of mean. The data were statistically analysed using one-way analysis of variance (ANOVA). Data from the histopathological test were compared with their respective controls and differences at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Phytochemical composition

Qualitative analysis of aqueous leaf extracts of *P. thonningii* confirms active phytochemicals such as alkaloids, tannins, anthraquinones, phenols, terpenoids, glycoside, saponins, flavonoids, steroids while phlobatannins is absent (table 2).

All the phytochemical constituents (table 2) identified in *Piliostigma* with previous work of Kwaji *et al* [17] and some of these phytochemicals have been linked to the capping and stabilizing ability of plant that have been used for nanoparticle synthesis [18].

3.2. UV-visible spectrophotometric of optimized biosynthesized silver nanoparticles

The UV-visible spectra (figure 2) indicate a strong surface plasmon resonance which is clearly visible at a peak of

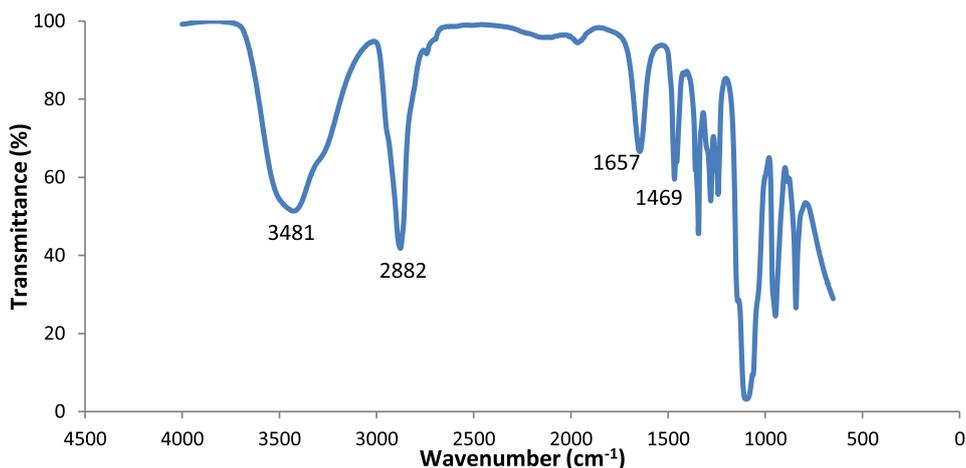


Figure 7. FTIR spectrum of biosynthesized silver nanoparticle SNP3.

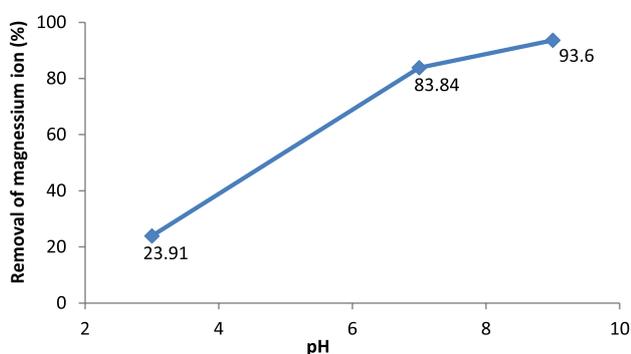


Figure 8. Removal percentage of magnesium ion using optimized biosynthesized silver nanoparticle SNP3.

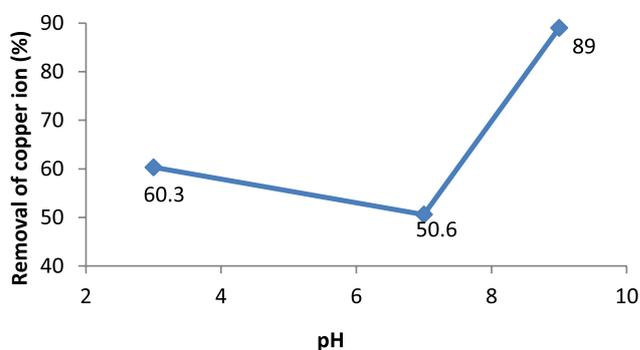


Figure 10. Removal efficiency of percentage of the removal of copper ion by optimized biosynthesized silver nanoparticle SNP3.

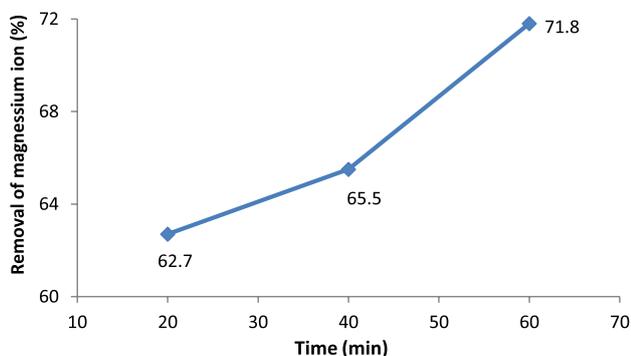


Figure 9. Removal percentage of magnesium ion at different contact time using optimized biosynthesized silver nanoparticle SNP3.

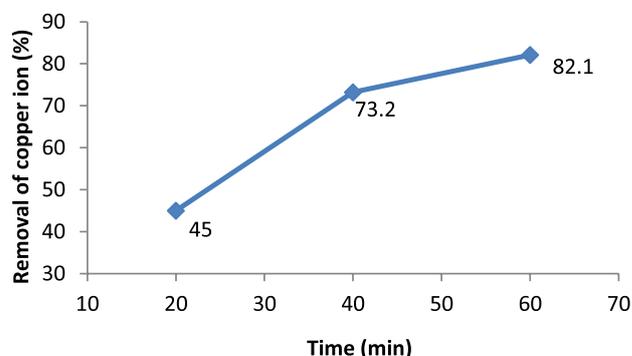


Figure 11. Removal efficiency of percentage of copper ion at different contact times by optimized biosynthesized silver nanoparticle SNP3.

411–415 nm. The optimized condition of SNP3 shows higher intensity compared to other conditions (SNP1, SNP2, SNP4 and SNP5).

The colour change with the absorbance at 411–415 nm wavelength for various condition of optimization agrees with previous work on silver nanoparticle green synthesis that range of wavelength is within 400–500 nm [7].

3.3. Particle size of biosynthesized selected silver nanoparticle SNP3

Figure 3 shows the particle size for biosynthesized silver nanoparticles SNP3. The condition for SNP3 gives the particle size range of 68.23–150.00 nm. Whereas the most intense particle size is 114.20 nm with percentage intensity of 13.6 (%) (figure 3).

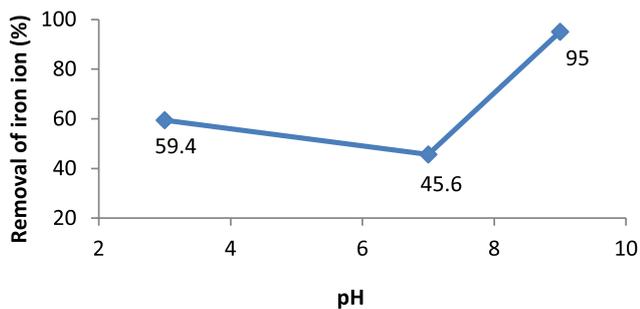


Figure 12. Removal percentage of iron ion by optimized biosynthesized silver nanoparticle SNP3.

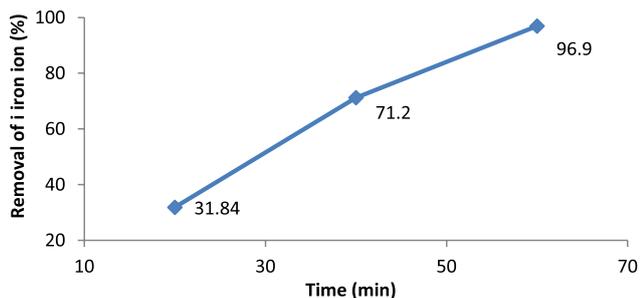


Figure 13. Removal percentage of iron ion at different contact time using optimized biosynthesized silver nanoparticle SNP3.

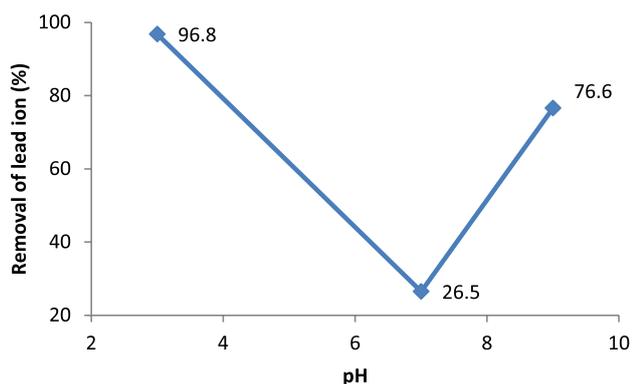


Figure 14. Removal percentage of lead ion at using optimized biosynthesized silver nanoparticle SNP3.

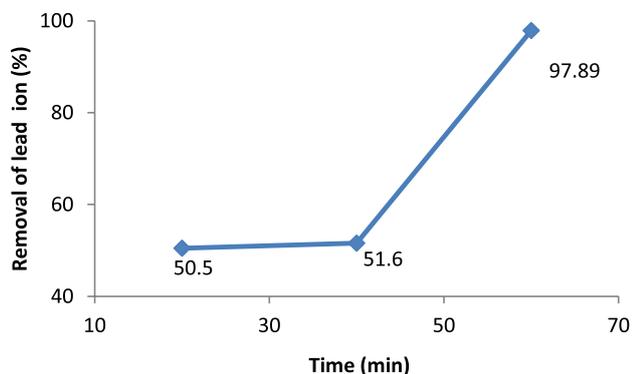


Figure 15. Removal percentage of lead ion at different contact time using optimized biosynthesized silver nanoparticle SNP3.

3.4. Energy dispersive x-ray spectroscopy of biosynthesized silver nanoparticle

The energy dispersive x-ray (EDX) spectrometry confirmed the presence of silver as shown in figure 4. The vertical axis shows the number of x-ray counts and the horizontal axis shows energy in keV. The EDX spectrum of optimized biosynthesized AgNPs (SNP3) shows an intense optical absorption band peak at 0.2, 0.5, 2.6 and 8.0 keV corresponding to carbon, oxygen, silver and copper respectively (figure 4). The presence of high count of other element such as carbon and copper may be from other component of the plants.

3.5. Transmission electron microscopy (TEM) and selected area electron diffraction (SAED)

The TEM images of SNP3 revealed spherical with various sizes of loosely bound at 50nm magnification (figure 5) while SAED ones confirmed the crystalline nature of the biosynthesized SNP3 (figure 6). The spherical shape with loose bound may be because of sonication treatment as reported by Dhand *et al* [7]. The strong presence of bright spot with crystal orientation at ring from SAED agrees with other work [7, 19].

3.6. Functional group composition of biosynthesized silver nanoparticle (SNP3)

FTIR spectral of SNP3 identify the major peaks at 2882 and 3481 cm^{-1} , the minor peaks at 1469 and 1654 cm^{-1} . The peak at 3456 cm^{-1} corresponds to $-\text{O}-\text{H}$ stretch which can be assigned to $-\text{O}-\text{H}$ stretch of hydroxyl groups. The peaks at 1469 and 1654 cm^{-1} corresponds to $-\text{C}=\text{C}$ stretching vibration (alkenes) and $-\text{NO}$ asymmetric stretch (nitro compounds) (figure 7). The presence of a major peak of hydroxyl group may be attributed to polyphenol compounds of the plant as reported that chemical tannins can act as a reducing agent for silver nanoparticle synthesis [20].

3.7. Atomic absorption spectroscopy (AAS) of simulated waste water

3.7.1. Effect of pH on removal of magnesium ion It was observed that the removal percentage was higher at pH 9 corresponding to 93.6% compared to pH 3 and 6 (figure 8).

3.7.2. The effect of contact time on removal magnesium ion The removal rate increases with time, so the optimal time is about 60 min with 71.8% best removal (figure 9).

3.7.3. The effect pH on copper ion removal The effect of pH was investigated on copper removal efficiency by biosynthesized silver nanoparticles in an initial concentration of 5 mg l^{-1} of copper ion (figure 10). It was observed that the removal efficiency percentage was higher at pH 9 (89%).

Table 3. Haematological profile of oral administration of silver nanoparticle treated water after 28 d.

Group	Hb (g/dl)	PCV (%)	MVC (Fi)	MCH (pg)	MCHC (g/dl)	RBC × 10 ¹² (l ⁻¹)	PLC × 10 ⁹ (l ⁻¹)	TWBC × 10 ⁹ (l ⁻¹)	N (%)	L (%)	E (%)
Control	10.26 ± 0.55	31.3 ± 1.45	52.3 ± 0.33	17.0 ± 0.0	32.3 ± 0.33	6.30 ± 0.11	147 ± 15.6	5.00 ± 0.63	9.50 ± 4.33	65.67 ± 9.82	24.33 ± 5.48
SNP3	7.80 ± 1.76	25.7 ± 4.33 ^a	50.0 ± 0.0	15.00 ± 0.57 ^a	30.67 ± 1.45	5.47 ± 0.72 ^a	203.6 ± 24.25 ^a	2.66 ± 0.84 ^a	30.33 ± 13.56	38.33 ± 13.56 ^a	31.0 ± 0.0
<i>p</i> value	0.143	0.002	0.263	0.014	0.291	0.01	0.00	0.007	0.001	0.003	0.008

Keys: N is neutrophils, L is lymphocytes, E is eosinophils.

Each value is of five determinations ±MSE. Values along the same column at *p*-value less than 0.05

^(a) are significantly different in comparison with control (*p* < 0.05).

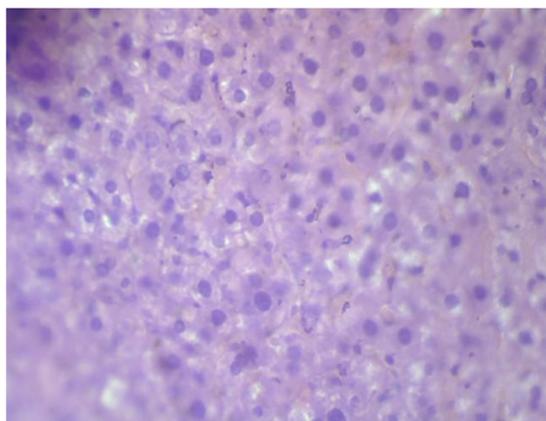


Plate 3. Micrograph of liver section of control group.

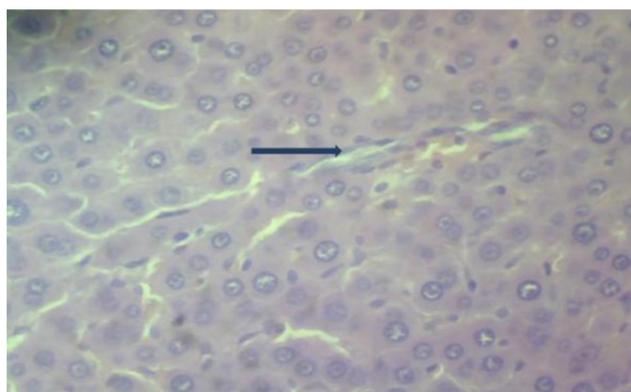


Plate 4. Micrograph of liver section of SNP3 group.

3.74. The effect of contact time on copper ion removal The effect of contact time was investigated on copper removal efficiency by biosynthesized silver nanoparticles in an initial concentration of 5 mg l^{-1} of copper at 20, 40 and 60 min. During this phase, adsorbent volume was 1 ml and the solution pH adjusted to 9. As observed, the removal rate increases with time and the optimal time is about 60 min with 82.1% for the best removal (figure 11).

3.75. The effect of pH on iron ion removal The effect of pH was investigated on iron ion removal efficiency by biosynthesized silver nanoparticles in an initial concentration of 5 mg l^{-1} of copper ion. During this phase, adsorbent volume was 1 ml and the solution pH adjusted to 3, 7 and 9. As observed, the percentage removal was higher at pH 9 (95%) (figure 12).

3.76. The effect of contact time on iron ion removal The effect of contact time was investigated on iron removal efficiency by biosynthesized silver nanoparticles in an initial concentration of 5 mg l^{-1} of iron at 20, 40 and 60 min. During this phase, adsorbent volume was 1 ml and the solution pH adjusted to 9. As observed, the removal rate increases with time so the optimal time is about 60 min (96.9%) for the best removal and this time was chosen to subsequent experiments (figure 13).

3.77. The effect of pH on lead ion removal The effect of pH was investigated on lead ion removal efficiency by biosynthesized silver nanoparticles in an initial concentration of 5 mg l^{-1} of lead ion. During this phase, adsorbent volume was 1 ml and the solution pH adjusted to 3, 7 and 9. As observed, the percentage removal was higher at pH 3 (96.8%) (figure 14).

3.78. The effect of contact time on lead ion removal The effect of contact time was investigated on lead ion removal efficiency by biosynthesized silver nanoparticles in an initial concentration of 5 mg l^{-1} of lead ion at 20, 40 and 60 min. During this phase, adsorbent volume was 1 ml and the solution pH adjusted to 9. As observed, the removal rate increases with time so the optimal time is about 60 min (97.89%) for the best removal and this time was chosen to subsequent experiments (figure 15).

3.8. Toxicity studies

3.8.1. Haematological studies The results of haematological studies are presented in table 3. There was a significant difference at $p < 0.05$ in the values of packed cell volume (PCV), red blood cell (RBC), total white blood cell (TWBC) and platelet counts (PLC) of the nanoparticle treated group in comparison with the normal control group. Whereas there was no significant difference at $p < 0.05$ in the values haemoglobin (Hb), mean cell volume (MCV) and mean cell haemoglobin concentration MCHC of the rats treated nanoparticle when compared to the control groups. The significant difference in the indices of anaemia characterised by the nanoparticle group may suggest cellular toxicity of the nanoparticle by prolonged uses which is in accordance with previous works of Kim *et al* [20], Adeyemi and Sulaiman [21].

3.8.2. Histopathological studies Plates 3 and 4 show the architecture organizations of the liver section of control and SNP3 groups, respectively. The control groups show that the hepatic architecture was preserved with congested blood vessels while the nanoparticle SNP3 shows a thickened thrombosed central vein and mild inflammation around the blood vessels. The inflammation occurrence with the nanoparticle treated group suggest cellular degenerate and rearrangement by long administration of the nanoparticle as reported by other researcher [21] and not in agreement with Armaghan *et al* [22].

4. Conclusion

From the study, it was observed that *P. thonningii* could reduce silver nitrate into its nanoparticle size with the influence of different experimental conditions resulting in different absorbance intensity but the same wavelength. The characterization of the responsible capping agent of the extract shows a likely link to phenolic (hydroxyl group) composition. The best optimization nanoparticles synthesized was effective in removal of heavy metals in laboratory simulated. And the safety evaluation of

nanoparticle treated water in an *in vivo* animal shows less toxic effects with no disruption of tissue distribution of AgNPs treated water after oral administration for 28 d.

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