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# Cytotoxicity Testing of Gold Nanoparticles from the Aqueous Extracts of *Sphagneticola trilobata* (L.) J.F Pruski on Shrimp Leach and Vero Cell line

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ABSTRACT

Nanotechnology in the field of biomedicine has developed rapidly in recent years. However, concerns about their safety as pharmaceutical agents prevent their widespread application. In this study, the toxicity properties were assessed for a gold nanoparticle product produced using *Sphagneticola trilobata* (L.) J.F. Pruski aqueous flowers and leaves extracts as a bio-reductor (AuNP-AFSt and AuNP-ALSt, respectively). Brine shrimp lethality (BSL) and Methythiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT) were used for the cytotoxic assay. Toxicity on *Artemia saline* Leach and Vero cells were stated as Lethal Concentration ($LC_{50}$) and Inhibitory Concentration ($IC_{50}$), respectively. The findings showed that the $LC_{50}$ values for AuNP-AFSt and AuNP-ALSt were 86.69 and 63.31 µg/mL. Additionally, the $IC_{50}$ for AuNP-AFSt and AuNP-ALSt were discovered at concentrations of 30.80 µg/mL and 5.09 µg/mL. These results indicated that AuNP-AFSt and AuNP-ALSt were toxic on Shrimp leach (Meyer's criteria), with moderate and highly toxic on Vero cells culture (NCI criteria).

***Keywords***: *Sphagneticola trilobata*; Gold nanoparticle, toxicity, *Artemia saline*, Vero cells

## Introduction

Nanotechnology in the field of biomedicine has developed rapidly in recent years.[1] This might be due to the superior characteristics of nanoparticles, such as their relatively stable nature compared to drugs prepared using conventional methods. Nanoparticles can also penetrate spaces between cells that colloidal particles can usually penetrate (high effectiveness in achieving target cell). In addition, nano-sized materials have a relatively larger surface that can promote better chemical reactivity, particularly in catalytic applications.[2] Nanoparticles can also be combined with other technologies, and their applications can be found in various fields, including agriculture, medicine, industrial, environmental, and renewable energy.[3]

The process of preparing nanoparticles, especially metal nanoparticles, has been directed towards using environmentally friendly methods for the last few years. This method is plant-based as a bio-reductor to produce metal nanoparticles.[4-6] One type of metal prepared in this way is gold nanoparticles. Gold nanoparticles have received great attention in recent years because they possess pharmacological activity. Apart from that, gold metal is also relatively non-toxic, and the synthesis process is relatively easy.[7-9]

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Green/ biological syntheses of gold nanoparticles have been carried out by several previous researchers using various plant extracts.[5,10-12] *Sphagneticola trilobata* (L.) J.F. Pruski has worldwide popularity because of its invasiveness and use as an ornamental plant. Previous studies reported the antioxidant, antibacterial, antifungal, and anticancer potential of this plant, and it could be a candidate for medicinal raw materials.[13-14]

The genus Sphagneticola has been developed into zinc, gold, and silver nanoparticles. For instance, *Wedelia urticifolia* (Blume) DC flower extract was used by Rather *et al*.[4] in the development of silver nanoparticles and revealed its antibacterial characteristics. Also, Das *et al*.[15] synthesized AgNPs utilizing *Wedelia chinensis* leaf extract as a bio-reductor, which exhibited antibacterial, antioxidant, and cytotoxic activity against some cancer cells. Again, Dey *et al*.[16] synthesized gold nanoparticles derived from *Wedelia trilobata* extract with cytotoxic activity against colon cancer cells. The above studies, notwithstanding, there is no literature report on the cytotoxic activity of gold nanoparticles using the flowers and leaves of *S. trilobata* as a bio-reductor on *Artemia leach* and Vero cell culture, although *Artemia leach* have been shown to exhibit similar biological responses with mammalian cells. Therefore, the cytotoxicity of gold nanoparticles (AuNPs) synthesized using *S. trilobata* extracts against shrimp leach and Vero cell lines was evaluated in this study, in order to determine its safety in humans. This is a preliminary study expected to contribute to the current knowledge base on the application of gold nanoparticles (AuNPs) as a pharmaceutical dosage form.

## Materials and Methods:

*Materials*

This investigation employed $HAuCl_4.3H_2O$ as the precursor solution and *Sphagneticola trilobata* (L.) J.F Pruski as a bio-reductor in the synthesis process. Other materials were Vero cell lines (ATCC CCL81), Fetal Bovine Serum, Penicillin-streptomycin, Dulbecco's Modified Eagle Medium, DMEM, Trypsin, and MTT. Others include a centrifuge, Orion Aquamate UV-Vis spectrophotometer, Biosafety

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Cabinet, inverted microscope, $CO_2$ incubator, T25 flask, and improved Neubauer 96-wells Tissue Culture Plate.

*Methods*

Preparation of the Precursor and Bioreductor Solution

A precursor solution ($HAuCl.3H_2O$) was prepared in a concentration of 1,000 ppm. An aliquot (394 ppm) of the precursor solution was used for the synthesis. The dried sample of S. trilobata was dissolved in an aqueous solution in a ratio of 1:9. The reductor solutions were prepared in condition of 60 $^oC$ for 30 minutes.[17]

*Biological Synthesis of Gold Nanoparticles (AuNPs)*

The reductor solutions consist of extracts from the flowers and leaves of *S. trilobata.* The reductor solution (100 mL) was added to 500 mL of the precursor solution (394 ppm). The reaction medium was maintained at 60ºC for 30 minutes. The gold nanoparticle solutions were purified by centrifugation at 6,000 rpm for 15 minutes.[16-17]

*Detection of AuNPs*

The colour change of the reaction medium confirmed the characteristic formation of the gold nanoparticles. The absorbance of the nanoparticle solution was measured in the wavelength range of 200-800 nm.[16]

Cytotoxic Activities of AuNPs

Cytotoxic activities of AuNPs were studied in vivo and in vitro assay using the Brine Shrimp Lethality (BSL).[18] and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide). [13-14]

*BSL Assay*

The *BSL* assay was carried out on Artemia salina leach. The BSL Test was performed in 5 mL seawater containing 10 leaches of *A. salina* aged 48 hours. The concentrations of the samples were 1000, 500, 250, 125, 62.5, and 32.25 µg/mL. The test tube was incubated for 24 hours at room temperature. Experiments were carried out in triplicate. The leach conditions were observed using a drop pipette illuminated by a lamp. The mortality of *A. saline* leach was recorded and stated as the percentage of mortality (formula 1), then analyzed using the linear regression approach. The $LC_{50}$ was determined by plotting the probit units against the corresponding log of concentration. A probit is a statistical probability measurement unit based on variances from the mean of a normal distribution. The observed $LC_{50}$ values are contrasted with the toxicity standards established by Meyer and Clarkson.[18-20]

$$\%\ Mortality = \frac{Number\ of\ dead\ larvae}{Number\ of\ tested\ larvae} x\ 100 \quad \text{.....[1]}$$

*MTT Assay.*

Furthermore, the cytotoxic assay was carried out on the Vero cell line (ATCC CCL81) using the MTT method. 100 µL of RPMI1640 was applied to grown Vero cells (±5,000 cells/well). The media was supplemented by (a) FBS (10%), (b) streptomycin (100 µg/mL) and (c) penicillin (100 U/mL). The samples were added after 50% confluent cell for 24 hours. On the 3rd day, 10 µL MTT (5 mg/mL) was added to each well and then incubated at 37$^oC$ for 4 hours. Ethanol was used for dissolving the formazan crystals. Absorption value readings were carried out at a wavelength of 595 nm.[14] The obtained data on the Vero cells line was reported as 50% inhibitory concentration ($IC_{50}$), calculated by plotting the probit units against the corresponding log concentration.[20]

## Results and Discussions

*Ultraviolet-Visible (UV-Vis) Spectrum of Gold Nanoparticles Formation*

The production of AuNPs using *S.trilobata* extract was confirmed by a colour change from clear yellow (precursor solution) to purple or reddish purple (Figure 1). This result was consistent with the synthesis of gold nanoparticles using *Garcia mangostana* fruit peels.[21] Similar observations in colour change (purple/deep purple colour formation) were reported in the synthesis of AuNP solution using *Limnophila rugosa* leaves, *Camellia sinensis,* and *Wedelia trilobata,* respectively.[22-23,16] who studied the AuNP solution using.

The UV-Vis instrument was used to characterize the gold nanoparticles formed by controlling changes in absorption values. The research demonstrated that the maximum UV peak was at 520 nm. This finding was in accordance with another study by Lee *et al.*[2], who observed the absorption peak of AuNP at wavelengths of 546 nm. Pirko *et al.*[23] observed the absorption peak of AuNP was at 550 nm.

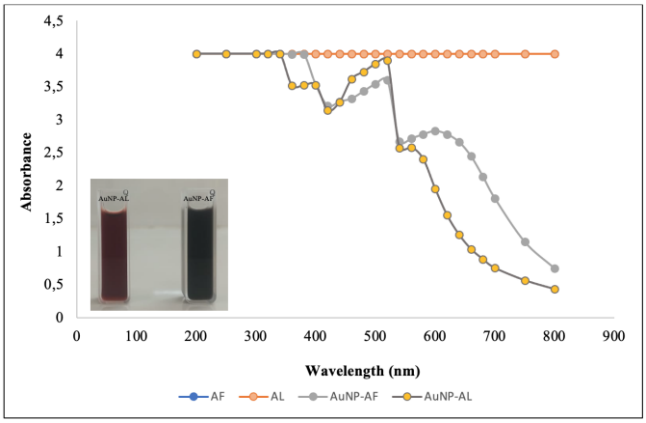


**Figure 1.** The curve of UV-Vis absorbance of the gold nanoparticles solution

*Toxicity Test of Gold Nanoperticle-S.trilobata Extract (AuNP-StE)*

A lethality assay was conducted using the BSL method. The relationship between the concentration of the samples and the mortality of artemia leach is shown in Figure 2. The percentage of leach mortality was concentration-dependent in the presence of extract alone or with AuNP. There statement was accordance to Sakhar *et al.*[24], who found a linear relationship between mortality and concentrations of test sample; an increase in percentage mortality with concentration. The cytotoxic activity of the experimental materials is reported as the half-lethal concentration ($LC_{50}$).

In this study, the 50% Lethality value is summarized in Table 1. The toxicity of AuNP-AFSt and AuNP-ALSt were 86.7 and 63.3 µg/mL, respectively. On the other hand, the flowers (AFSt) and leaf extracts (ALSt) exhibited $LC_{50}$ values of 54.6 and 55.8 µg/mL, respectively. From these results, the flowers and leaves extracts of *A. trilobata* are more toxic compared to AuNP-AFSt and AuNP-ALSt. Meyer's toxicity index (1982) showed that $LC_{50}$ < 30 µg/mL is highly toxic, while 31 µg/mL < $LC_{50}$ < 100 µg/mL is toxic and $LC_{50}$ > 1000 µg/mL is not toxic. According to Meyer's criteria, the results were categorized as toxic and could have a potential for anticancer activity.[19]

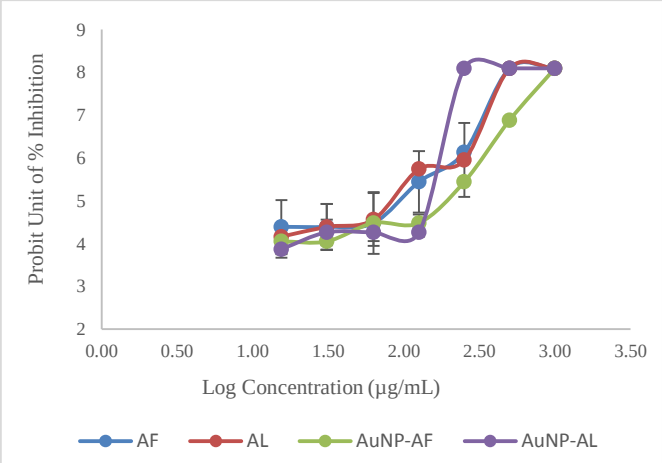


**Figure 2.** The relationship between the (lethal) concentration of the samples on the mortality of shrimp leach *A. salina*

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**Table 1:** Lethal concentration ($LC_{50}$) of the samples

| Sample | Equation | $R^2$ | Value ($LC_{50}$) (µg/mL) | Toxicity class (Meyer criteria) |
|---|---|---|---|---|
| AF | Y = 2.3898x + 0.8476 | 0.8829 | 54.638 | Toxic |
| AL | Y= 2.4409x + 0,7362 | 0.9052 | 55.821 | Toxic |
| AuNP-AF | Y = 2.2226x + 0,6925 | 0,8502 | 86.696 | Toxic |
| AuNP-AL | Y = 2.8643x - 0.1602 | 0.7842 | 63.314 | Toxic |

**Table 2:** The 50% Inhibitory concentration ($IC_{50}$) of the samples

| Sample | Equation | $R^2$ | Value ($IC_{50}$) (µg/mL) | Toxicity class (NCI criteria) |
|---|---|---|---|---|
| AuNP-AFSt | Y = 1.4846x + 2.79 | 0,8939 | 30.80 | Moderate toxic |
| AuNP-ALSt | Y = 0.7015x + 4.5035 | 0.9626 | 5.09 | Highly toxic |

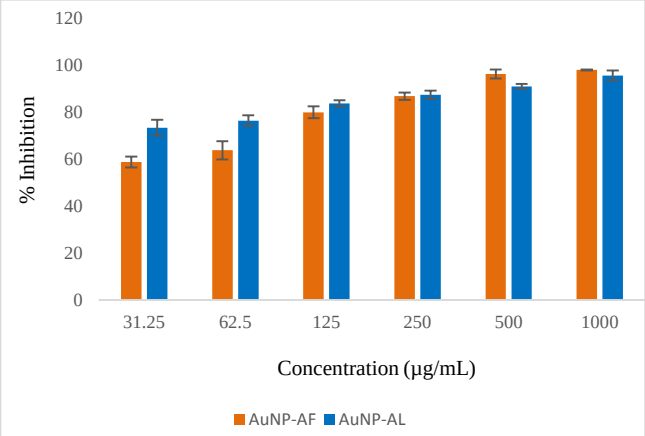


**Figure 3.** Cytotoxic activities of the AuNPs on Vero cell lines

Results of the *in vitro* cytotoxicity of the synthesized AuNP-AFSt and AuNP-ALSt on Vero cell lines are shown in Figure 3. The result demonstrated significant toxicity even at the initial concentration of 31.25 µg/mL. The $IC_{50}$ values of AuNP-AFSt and AuNP-ALSt were calculated using probit analysis (Table 2) with a half-maximal cytotoxic concentration of 30.80 µg/mL and 5.09 µg/mL, respectively. Similar results were reported by Meléndez-Villanueva *et al.* [25], who synthesized gold nanoparticles using garlic extract and discovered an effective concentration ($EC_{50}$) of 8.829 µg/mL against Vero cells.
Geran's protocol and the U.S. National Cancer Institute (NCI) classified substance or compound toxicity as follows: (a) a substance is highly toxic when $IC_{50}$ value <20 µg/mL, (b) moderately toxic when the $IC_{50}$ value is in the range of 21 - 200 µg/mL, (c) weakly toxic if $IC_{50}$ value in the range of 201 – 500 µg/mL and (d) not toxic when $IC_{50}$ value is more than 501 µg/mL. According to these criteria, the AuNP-AFSt and AuNP-ALSt were categorized as moderately toxic and highly toxic, respectively, against Vero cell culture.

## Conclusion

In this study, the gold nanoparticle was synthesized biologically using the aqueous extracts of *S. trilobata* leaves and flowers (denoted AuNP-AFSt and AuNp-ALSt, respectively) at 60°C for 30 minutes. The toxicity testing revealed that the AuNP-AFSt and AuNP-ALSt were cytotoxic against Vero cell lines at $LC_{50}$ values of 86.69 µg/mL, 63.31 µg/mL, and 30.80 µg/mL, 5.09 µg/mL, respectively. From these findings, AuNP-AFSt and AuNP-ALSt were categorized as toxic against shrimp leach and moderately toxic against the Vero cell line according to Meyer's criteria. This study shows that *A. trilobata* gold nanoparticles could be a potential source of pharmaceutical lead in the development of anticancer agents.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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