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Green Synthesis of Sliver Nanoparticles Using *Costus Afer* Aqueous Leaf Extract and its Effect on Liver and Kidney Biomarkers in Adult Male Wistar Rat

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ABSTRACT

Because nanotechnology has so many uses in waste management, solar energy, catalysis, and sensing, it is a field of study that is increasingly gaining attention from scientists. In the field of medicine, nanomaterials are effectively employed for wound healing, medication delivery, cardiovascular disease diagnostics and therapy, and the creation of antibacterial agents. The purpose of the current study was to assess how *Costus afer*-AgNPs extract affected male rats' liver indicators. There were eighteen adult male rats utilized in this experiment. Six groups of rats were randomly assigned. Throughout the experiment, the first group (control) ingested enough water and compressed food without any limitations. The *Costus afer*-AgNPs extract was taken daily by the second, third, fourth, fifth, and sixth experimental groups, who took 200, 400, 600, 800, and 1000 mg/kg body weight, respectively. Four weeks later, all groups had blood drawn under anesthesia. They separated the serum. ALP, ALT, and AST concentrations in the serum were determined, and uric acid, creatinine, and urea were examined. The LSD test, ANOVA, and SPSS were used to evaluate the gathered data. Results showed a significant increase in the mean serum urea and uric acid levels of rats but significantly brought back the markers to normal after treatment with an increasing dose of the extract. Serum levels of AST, ALP, and ALT significantly increased in the experimental groups receiving 200mg, 400mg, 600mg, 800mg and 1000mg *Costus afer*-AgNPs extract per kilogram body weight compared to the control group ($P<0.05$). *Costus afer*-AgNPs could be toxic upon consumption even at increasing doses.

KEYWORDS: *Costus afer*, Medicinal plant, Liver, Kidney, Nanoparticles

INTRODUCTION

Nanotechnology is a science centered on atomic, molecular, and supramolecular molecules aiming to create nanostructures with enhanced functionalities, and the term nanoparticle describes particulate matter ranging in size from 1–100nm. Bearing a nano-scale size offers the advantage of having a significantly large surface area to volume ratio. The enhanced physical and chemical properties of nanoparticles, which find applications in diverse fields like antimicrobial development, bio-molecular detection, diagnostics, catalysis, micro-electronics, sensing devices, and drug targeting to cancer cells, are attributed to their increased surface area in conjunction with their conformation and distribution in solution (Moodley *at al.*, 2018). Green synthesis is defined as the use of environmentally compatible materials such as bacteria, fungi and plants in the synthesis of nanoparticles (Patra and Baek, 2014). These alluring green techniques are environmentally beneficial as they don't have the drawbacks of traditional synthetic strategies. (Veerasamy *et al.*, 2011). On the other hand, synthesis from biologically generated extracts has a number of benefits, including quick synthesis, large yields, and—most importantly—the elimination of the need for expensive downstream processing to create the particles (Gannimani *et al.*, 2014; Das *at al.*, 2014). Hence, nanoparticle synthesis from plant extracts tentatively offers a route for large-scale production of commercially attractive nanoparticles.

Costus afer (*C. afer*) is a plant commonly known as ginger lily, spiral ginger, or bush cane. Traditional medicine practice (TMP) claims to utilize it to cure and manage a variety of conditions, such as gout, diabetes mellitus, stomachaches, arthritis, and inflammation (Boison *et* al., 2019). These alleged ethnomedical use have prompted numerous investigations on the plant to gather scientific data. According to the Boison et al. (2019) search report, the plant's stem and leaves are rich sources of both macro- and micronutrients. Several steroidal sapogenins, aferosides, dioscin, paryphyllin C, and the flavonoid glycoside kaempferol-3-O-α-L-rhamnopyranoside are found in the leaves, stem, rhizomes, and roots of C. afer . Analgesic, anti-arthritis, antibacterial, antioxidant, CNS depressant, hepatocellular protection, cardioprotection, nephroprotection, testicular protection, and antihyperglycemic were among the bioactivities found in experimental tests conducted on different portions of the plant (Boison *et* al., 2019).

Due to toxic chemicals, conventional physical and chemical methods presently have limited use in preparing metal nanoparticles (Bhattacharya and Mukherjee, 2008). Additionally, these techniques are linked to significant energy input and expensive downstream processing (Awwad *et al.*, 2013). In

addition to their distinct treatment methods, nanobiotechnology and its byproducts are unique in their particle sizes, physical, chemical, and biological characteristics, and their wide variety of uses. Due to a lack of novel technique implementation on a big industrial scale, the current developing field of nanobiotechnology is still in its early stages of growth and has to be upgraded with contemporary technologies. (Jalab *et al.*, 2021). The report has validated that the physical method of synthesis uses techniques such as gas phase deposition, laser burning and mechanical grinding to synthesize nanoparticles with the advantages of simpler principles and higher purity, but the particle size is larger than the synthesized one by chemical and biological methods (Jalab *et al*., 2021). The chemical method requires external stabilizer agent to protect nanoparticles from aggregation and reducing agents to reduce Agþ to Ag0 to synthesize silver nanoparticles such as sodium citrate and sodium borohydrate (Jalab *et al*., 2021). By synthesizing silver nanoparticles utilizing a variety of biological agents, including yeasts, enzymes, bacteria, polysaccharides, algae, oligosaccharides, fungi, DNA, and human cell lines, the biological techniques are able to overcome the majority of these disadvantages. Given the accessibility, safety, and abundance of metabolites found in medicinal plants, plant extracts have the potential to function as both stabilizing and reducing agents for metal nanoparticles throughout the reduction process . The aqueous extract of *Costus afer* synthesizing Ag nanoparticles (extract Ag nanoparticles) is important to be investigated mainly because it can be used as a potential medicine for liver, and kidney and can be packaged in the form of infusions/ fluids. Liquids that use water solvents are much safer for health and the environment than using chemical solvents. The aim of the study was to carry out the green synthesis of silver nano particles using *Costus afer* aqueous leaf extract and its effect on liver and kidney biomarkers of adult male wistar rat.

MATERIALS AND METHODS

Materials

Plant Materials

In Umuaduru, Osisioma L.G.A. in Abia State, Nigeria, a farm yielded the leaves of *Costus afer*. Dr. Duru, C.N. of Environmental Biology at Federal Polytechnic Nekede, is a botanist who recognized the plant sample.

Animals

In this study, adult male rats were employed. These animals were bought from a nearby breeder in Imo State's Ihiagwa Owerri-West L.G.A. The Department of Biochemistry's animal house housed the animals in well-ventilated stainless steel wire cages. After purchase, the rats were fed a regular diet for at least two weeks to help them get used to the lab setting.

Chemicals and Reagents

Chemicals

All chemicals to be used in this study were of good analytical grade.

Methods

Preparation of plant material and Extraction

Locally grown, fresh, healthy *Costus afer* leaves were gathered, chopped into little pieces, and allowed to dry at room temperature after being thoroughly cleaned with tap water and then distilled water to get rid of all the dust and undesired visible particles. After weighing out roughly 10 g of the powdered plant sample, it was put into a 250 mL beaker with 100 mL of distilled water and allowed to boil for about 20 minutes. To eliminate particle debris and obtain a clear solution, the extract was filtered three times through Whatman No. 1 filter paper. The clean solution was then stored in 250 mL Erlenmeyer flasks at 4°C for subsequent studies. Sterility conditions were upheld throughout the entire experiment to ensure the efficacy and accuracy of the results without contamination.

Synthesis of Silver Nanoparticles (Ag-np)

The green synthesis of Ag-np was prepared following the method reported in the literature (Khan *et al*., 2018). Preparation was done by reacting 10 mL of the *Costus afer* leaf extract with 90 mL AgNO3 solution (1 mM) and was agitated on the air bath magnetic stirrer for 15 minutes at room temperature. A color change was observed from colorless to pink. The mixture was centrifuged and dried in the oven at temperature between 50°C – 60°C overnight

Experimental design

The experimental animals were randomized into 6 groups of 3 rats each and treated as follows;

Group 1: The rats in this group served as control and were given a normal diet and distilled water.

Group 2: The rats in this group were given an oral dose of 200 mg/kg (Low Dose) of the synthesized nanoparticle of *Costus afer* mediated AgNps.

Group 3: The rats in this group received oral gavage every day for 28 days at a dose of 400 mg/kg (low dose) of the synthesized nanoparticle of *Costus afer* mediated AgNps.

Group 4: Rats in this group received oral gavage every day for 28 days at a dose of 600 mg/kg (High Dose) of the synthesized nanoparticle of *Costus afer* mediated AgNps.

Group 5: The rats in this group received an oral gavage every day for 28 days at a dose of 800 mg/kg (High Dose) of the synthesized nanoparticle of *Costus afer* mediated AgNps.

Group 6: The rats in this group received an oral gavage every day for 28 days at a dose of 1000 mg/kg (High Dose) of the synthesized nanoparticle of *Costus afer* mediated AgNps.

Body weight and organ weight measurements

The rats' organ weights were measured using a Top loader weighing balance at the conclusion of the trial, following sacrifice, while their body weights were measured once a week.

Sacrifice of animals

After 28 days, each rat had a transverse incision done in the ventral wall of its abdomen while it was somewhat sedated with chloroform. Additionally, blood samples were taken from the descending abdominal aorta and homogenized in a simple container in order to estimate biochemical assays. .

Assay of alanine amino transferase (ALT) activity

Serum ALT activity was estimated by the method of Reitman and Frankel (1957)

Assay of serum aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) activity was determined according to the method of Reitman and Frankel (1957).

Assay of serum alkaline phosphatase (ALP) activity

The activity of alkaline phosphatase (ALP) was assayed using the method of Kochmar and Moss (1976).

Assay of Serum Urea

Urea concentration was determined using the method of Bartels and Bohmer (1972) as described in Randox Kit.

Assay of serum Creatinine

The serum creatinine was determined using the method of Bartels and Bohmer (1972) as outlined in Randox kit.

Determination of Serum uric acid

Serum uric acid level was determined using uricase method as described by Trivedi et al., (1978).

Statistical analysis

Statistical analysis was carried out using SPSS version 23 for Windows (IBM Statistics for Social Sciences). One-way analysis of variance (ANOVA) followed by Duncan's posthoc test for multiple comparisons was performed to determine differences between treatment groups. A p-value less than 0.05 was considered statistically significant. Results were expressed as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

1.1. Effect of Costus afer-AgNps on liver biomakers of experimental rats.

Table 1.1.: Result showing the *Costus afer*-AgNps on liver indices of experimental rats

Groups	No of Rats	AST	ALP	ALT
Normal Control	3	25.50 ± 0.70^{a}	23.50 ± 0.70^{a}	142.50 ± 0.70^{f}
200mg/kg bwt	3	30.10 ± 0.14^{b}	31.50 ± 0.70^{b}	104.50 ± 0.70^{c}
400mg/kg bwt	3	41.50 ± 0.70^{e}	35.50 ± 0.70^{c}	96.50 ± 0.70^{b}
600mg/kg bwt	3	39.50 ± 0.70^{d}	30.50 ± 0.70^{b}	85.50 ± 0.70^{a}
800mg/kg bwt	3	46.50 ± 0.70^{f}	41.50 ± 0.70^{d}	111.50 ± 0.70^{d}
1000mg/kg bwt	3	37.50 ± 0.70^{c}	40.50 ± 0.70^{d}	105.50 ± 0.70^{c}

n= 3. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts across columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across columns are considered non-significant ($p > 0.05$)

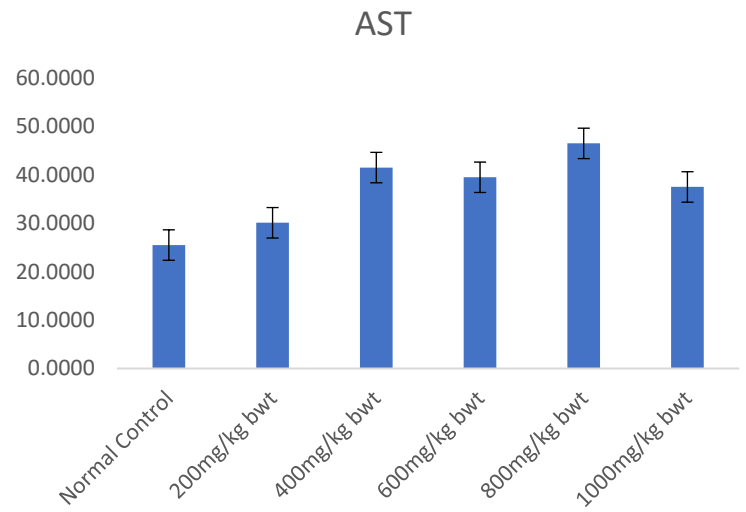

Figure 1: Graph showing the effect of *Costus afers*-AgNps on the AST of experimental rats

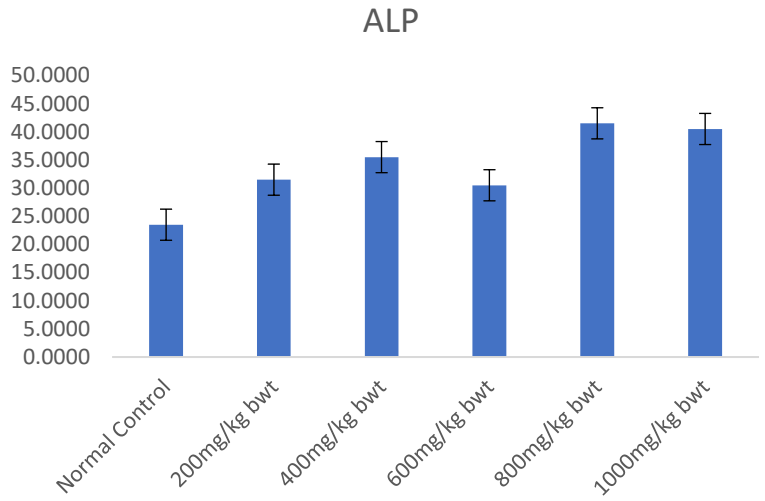

Figure 2: Graph showing the effect of *Costus afers*-AgNps on the ALP level of experimental rats

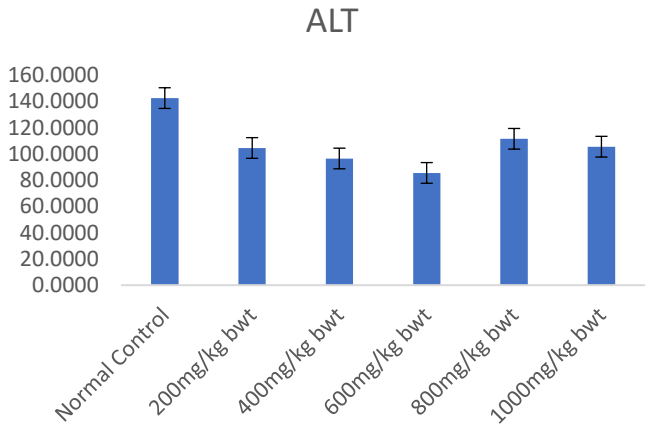

Figure 3: Graph showing the effect of *Costus afers*-AgNps on the ALT level of experimental rats

1.2. Results showing the effect of Costus afer-AgNps on Kidney of experimental rats.

Table 1.2.: Result showing the *Costus afer*-AgNps on kidney indices of experimental rats

Groups	No of Rats	Creatinine	Urea	Uric
Normal Control	3	1.35 ± 0.29	24.50 ± 0.29	3.25 ± 0.29
200mg/kg bwt	3	1.60 ± 0.29	26.00 ± 0.29***	4.45 ± 0.29**
400mg/kg bwt	3	1.80 ± 0.29	30.00 ± 0.29****	5.05 ± 0.29****
600mg/kg bwt	3	2.25 ± 0.29*	32.00 ± 0.29****	3.25 ± 0.29
800mg/kg bwt	3	2.24 ± 0.29**	30.50 ± 0.29****	3.45 ± 0.29
1000mg/kg bwt	3	1.85 ± 0.29	28.50 ± 0.29****	3.65 ± 0.29

n = 3. *P<0.05, **P<0.01, ***P<0.001. Values with superscripts *, **, *** are significantly different within the column on comparison to the control group while values with no superscripts are non-significantly different (p>0.05).

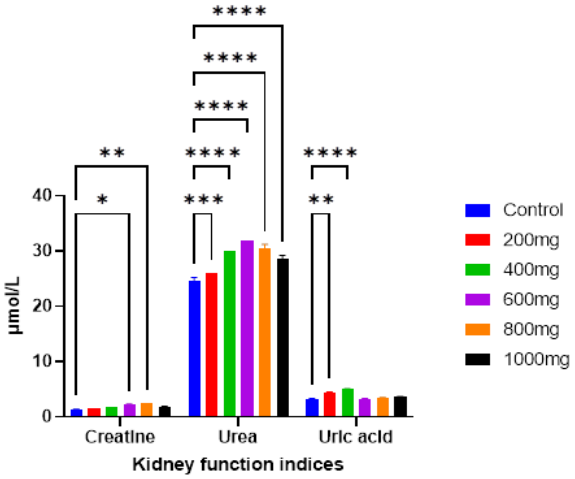

Figure 4: Bar chart showing the kidney function indices of experimental rats treated with *Costus afer*-AgNps

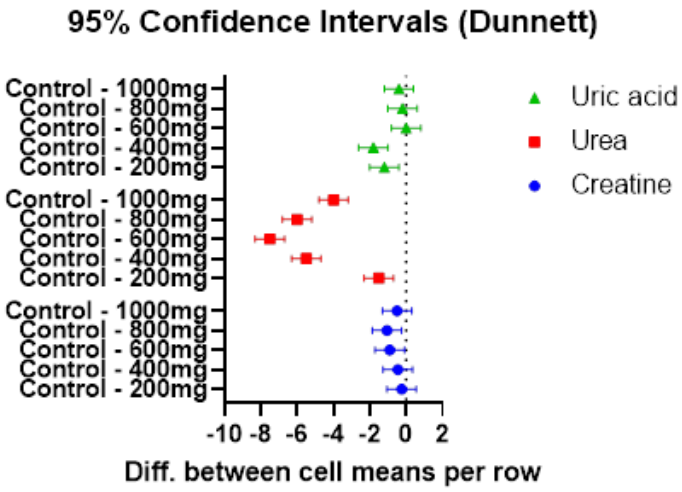

Figure 5: Forest plot showing the kidney function indices of experimental rats treated with *Costus afer*-AgNps. 95% Cls crossing the line of no effect are non-significantly (p>0.05) different while 95% Cls not crossing the line of no effect are significantly (p<0.05) different.

Discussion

An unfavourable interaction between the major organs and the plant extract, which is typically represented in the organ-to-body ratio, would lead to cellular constriction and inflammation (Kuatsienu *et al*., 2017). Two significant mammalian organs that are essential to the body's various metabolic functions are the liver and kidneys. "Detoxification, bile excretion, glycogen and vitamin storage, protein synthesis (fibrinogen, albumin, and globulin), blood clotting factor synthesis (fibrinogen, prothrombin, factor V, VII, IX, X, and XI), lipid metabolism (synthesis of cholesterol, phospholipid, endogenous triglycerides, and lipoproteins), and elimination of exhausted cells and microorganisms by phagocytosis by Kupfer cells are among the tasks assigned to the liver" (Adebiyi *et al*., 2021). There is a tendency for one or more of these tasks to be disrupted where and when this robust organ is attacked or destroyed. This could alter metabolic processes and have severe repercussions on the entire system, resulting in issues and eventually death.

The living system's homeostasis is preserved by the liver. It participates in the biochemical processes required for development and illness prevention (Ward and Daly, 1999). Many medicinal uses for the Costus afer plant give rise to questions regarding its safety and potential toxicity. This study suggests that there may have been hepatotoxicity, as evidenced by the decreased serum total protein concentrations. Toxicants in the phytochemical constituents of the leaves may have caused this, or there may have been an increase in the release of tissue-specific enzymes and other intracellular proteins as a result of disruption of the cell membrane caused by parasites.

The liver is one of the organs that is typically impacted by xenobiotic consumption. Hepatic damage is commonly linked to changes in the levels of certain liver and serum enzymes, such as ALT, AST, and ALP (Antai *et al*., 2009). Research utilizing extracts from medicinal plants has demonstrated the diverse impacts of phytochemicals on liver and serum enzyme levels. Certain phytochemicals are protective to the liver, whereas others are hepatotoxic (Antai *et al*., 2009). As the primary organ for material metabolism, secretion, and disposal, the liver is also involved in most biochemical pathways that support growth, disease prevention, nutrition supply, energy production, and reproduction. It plays a critical role in establishing, implementing, and regulating homeostasis in the body. Many academics feel that the existence of life is connected to the development of all diseases or the improper operation of different body parts (Tahmasebi et al., 2018). The liver's ability to aid in the metabolism of digestible substances, such as food, dietary supplements, alcohol, and the majority of medications, is one of its basic and key roles. Drugs can harm the liver in a number of ways, from minor abnormalities like elevated serum aminotransferase activity to serious organ destruction including intraperitoneal cholestasis or hepatic necrosis (Tahmasebi et al., 2018). The findings of this study revealed that at increasing doses, the extract was significantly different and higher for AST and ALP ($p<0.05$) from the control group which suggests that the extract could be toxic and the extract was significantly ($p<0.05$) lower in the ALT levels of the test.

Due to its high blood flow and ability to filter huge volumes of toxins, which can concentrate in the kidney tubules, the kidney is extremely vulnerable to toxicants (Akanji *et al*., 2013). Because of reports of illnesses and deaths, especially nephrotoxicity, the safety of using herbal medicines has recently come under scrutiny (Park *et al*., 2010). Elevated blood urea nitrogen and creatinine levels may indicate a kidney-related underlying disease (Raj, 2014). This study examined the dose-dependent effects of synthetic nanoparticles derived from Costus afer on urea, creatinine, and uric acid. . The result as shown in Table 1.2 revealed that there was a significant ($p < 0.05$) increase in the creatinine levels of the rats treated with a dose of 600mg/kg and 800mg/kg compared to the control and other test groups. The Dunnet 95% Cl revealed that urea levels of rats treated with different doses of the nano-synthesized extract were all significantly ($P<0.05$) higher when compared to the control group. At a dose of 200mg and 400mg/kg, uric levels were significantly higher ($P<0.05$) in comparison to the control and other groups. Due to its high blood flow and ability to filter huge volumes of toxins, which can accumulate in the renal tubules, the kidney is extremely vulnerable to toxicants (Lawal *et al*., 2015). Systemic toxicity from nephrotoxicity can lead to a reduction in the body's capacity to eliminate waste products, an imbalance in bodily fluids and electrolytes, and a reduction in the production of vital hormones (Lawal *et al*., 2016). Consequently, urea, creatinine, and uric acid measurements are crucial for figuring out the kidney's synthetic and excretory functions (Onukogu *et al*., 2019; Yusuf *et al*., 2018).

The skeletal muscle uses creatinine, which is the catabolic result of creatinine phosphate. It's a muscle metabolite called creatinine, and the body's muscle mass determines how much of it is in the serum. Creatinine levels are typically steady. Because of its easy excretion by the kidneys, higher levels are indicative of impaired renal function (Umar *et al*., 2019). As the final byproduct of protein metabolism, urea indicates the amount of protein in the diet and the rate at which protein is catabolized (Akanji *et al*., 2013). It is a waste product that remains after protein is broken down. Before the kidneys filter it out and eliminate it in the urine, urea circulates in the blood (Latha *et al*., 2016). There will be elevated blood urea levels if the kidneys are not working adequately. Serum urea and uric acid concentrations rise due to a compromised nitrogen balance and decreased protein synthesis (Umar *et al*., 2019), a sign of increasing renal impairment. .Following the administration of the extracts, there was a non-significant ($p > 0.05$) increase in serum creatinine at doses of 200 and 400 mg/kg, which suggests a functional renal capacity (Yusuf et al., 2018). Reduced protein catabolism or renal failure could be the cause of the notable change in serum urea after the extracts were administered (Lawal et al., 2015).

Conclusion

The compounds contained in extracts of *Costus afer* leaf mediated with synthesized silver particles increased some of the liver and kidney biomarker enzymes and caused some significant alterations in some of those biomarkers. In summary, the AgNps mediated by *Costus afer* may be harmful and toxic to the liver enzymes when consumed, leading to liver damage. When used for an extended period of time and at a higher dosage, it may also be hazardous to the kidneys. Further research should be done to characterize and identify the chemicals in the AgNPs mediated with Costus afer, based on the results of this work. Furthermore, it is necessary to look into the safety degree of *Costus afer* mediated by AgNps.

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