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Research Paper

Green Synthesis, Characterization and Phytochemicals Analysis of Silver Nano-Particles Using Aqueous Peel Extract of *Cucumis sativus*.

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Abstract
Currently, metal nano-particles have shows various application in medicine, pharmacy, and agriculture. Nanobiotechnology, in combination with green chemistry, has great potential for the development of novel products that are beneficial to human health, environment as well industries. In this study, silver nano-particles (AgNPs) were synthesized using aqueous peel extracts of Cucumis sativus by eco-friendly, inexpensive and nontoxic biological methods. The silver precursor used was silver nitrate solution. A visual observation was used to confirm the formation of silver nano-particles while the characterization of the synthesized silver nano-particle was carried out using UV-Visible spectroscopy and Transmission Electron Microscopy (TEM). The phytochemicals screening were also conducted to confirm the constituents of the synthesized Cucumis sativus peel silver nano-particle (CP-AgNPs). A visible colour changes was observed after 1 h from yellowish to reddish brown and became dark brown after 5 hs for 1 mM, 3 mM and 5 mM $AgNO_3$ solution at 1:1 (v/v) to the extract, the color was more darker at 5 mM $AgNO_3$ and the UV-Vis spectroscopy at a fixed 5 mM AgNO3, showed surface plasmon resonance with maximum absorbances of 434 nm, 489 nm and 522 nm for the nanoparticles obtained at three (3) different concentration of 2,5 and 20%(w/v) of the Cucumis sativus peel extract respectively. The TEM showed a spherical shape with no aggregate and the visualization using Image J software, were found to be about 45 nm, 115 nm and 47 nm with polydispersity of 32.1%, 10.8% and 46.0%, for CP-AgNPs at concentration of 2,5 and 20%(w/v) respectively. The phytochemicals screening revealed the presence of tannins, saponins, flavonoids, glycosides, steroids and Terpenoids which are responsible for the reducing and capping property of CP-AgNPs .
Key words: *Cucumis sativus, Cucumber Peel, Aqueous Extract, Silver, Nano-particles, Phytochemicals*

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I. INTRODUCTION

Agriculture accounts for roughly 40% of Nigeria's GDP, over 65 percent of employment, and a significant portion of non-oil revenue. Unfortunately, it faces numerous problems, including post-harvest losses as a result of insufficient processing and storage facilities. This has undoubtedly resulted in a rise in the number of agricultural wastes generated as a result of such losses, as well as by-products of processing and consumption. Mangoes, watermelons, sweet potatoes, cucumber, tomotoes and Irish potatoes are among the agricultural products in Nigeria that contribute to the contamination of our local surroundings. They are cultivated in vast quantities, and some even grow wild, and are consumed in large quantities, resulting in waste products that litter our environment due to poor waste management techniques, and making their by-products freely available. As a result, there is no financial means or motivation to invest in extending the shelf life of these fruits an tubers, either through improved production or processing.

Nanotechnology is a rapidly growing field of current study that involves the synthesis, design, characterization, manufacture, and use of structures, devices, and systems at the nano scale by controlling shape and size [1]. Nanotechnology also entails the production of nanoparticles ranging in size from 1 to 100 nanometers [2]. Due to their diverse qualities, nanoparticles can be used in a variety of applications, including medicine, pharmaceuticals, manufacturing and materials, environmental, electronics, energy gathering, and mechanical sectors [3] . Carbon nanotubes, quantum dots, nanorods, nanocapsules, nanoemulsions, fullerenes, metallic nanoparticles, ceramic nanoparticles, and polymer nanoparticles are all examples of nanoparticles [4].

Regarding the metallic nano-particles, their outstanding properties have caused the development of different methodologies for their synthesis, where gold (Au) and silver (Ag) nano-particles prepared from plant extracts are of great interest for the researchers in their attempt to develop suitable antimicrobial agents for agriculture [5]. Besides, these initiatives are considered as low-cost processes that allow avoiding toxic-generating products and benefit the agricultural activity. Silver nanoparticles (AgNPs) are used as antimicrobial agents in catheters to prevent infections during surgery and similarly possess anti-fungal, anti-inflammatory, anti-angiogenic, and anti-permeability properties. Silver is also one of the components in creams production for healing wounds [6]. However, silver nano-particles are now being introduced as an alternative antibacterial agent replacing silver ions. Both silver ions and silver nano-particles have inhibitory and lethal effects on bacterial species such as *Escherichia coli*, *Staphylococcus aureus*, and even yeast [7]. In addition to antibacterial activity of the silver nano-particles, a complete disruption of the bacterial membrane of *Escherichia coli* cells was observed after few minutes in contact with silver nano-particles under TEM analysis [8]. The high efficiency AgNPs is mainly due to the availability of larger surface area to volume ratio for interactions, easing the penetration and disruption of nano-particles into the bacterial cells, as compared to micro-sized silver ions [9].

The cucumber is a member of the *Cucurbitaceae* family, along with squash and different kinds of melon. Cucumbers are high in water and low in calories, fat, cholesterol, and sodium. They have a mild, refreshing taste and high water content. They can be refreshing and pleasant to eat in hot weather and help prevent dehydration. It is eaten savory, but it is strictly a fruit. *Cucumis sativus* have been grown in Nigeria, India etc for food and medicinal purposes since ancient times, and they have long been part of the Mediterranean diet. *Cucumis sativus* consist mainly of water [10]. *Cucumis sativus* are believed to have anti-inflammatory benefits. Used directly on the skin, sliced cucumber has a cooling and soothing effect that decreases swelling, irritation, and inflammation. It can alleviate sunburn. Placed on the eyes, they can help decrease morning puffiness.

This study is aims to synthesized and characterized silver nano-particles using *Cucumis sativus* peel aqueous extract and also to screened phytochemicals components of the synthesized silver nano-particles.

II. METHODOLOGY

2.1. Chemicals

All the reagents purchased were of analytical grade, Silver nitrate (AgNO) and distilled water were purchased from Sigma-Aldrich with a =99.5% purity.

2.2. Collection of *Cucumis sativus* peel

Cucumis sativus peels were obtained from Kazaure farms land area, Jigawa state, Nigeria.

Figure 1. *Cucumis sativus* peel(a) and fruit (b)

2.3. Preparation of aqueous peel Extract.

The Aqueous peel extract of *Cucumis sativus* was prepared by the following procedure: fresh peels of *Cucumis sativus* was collected and washed with tap water at first, and then the surface was washed with distilled water until no impurities remained, the fresh peels were cut into small pieces, and 20 g each was weighed and put into a beaker with 200 ml of distilled water. Mixture was heated for 20 minutes at 60^oC and was occasionally stirred and it was allowed to cool at room temperature [11]. Mixture was filtered using the Whatman 42 filter paper and then centrifuged at 81 G-force for 20 minutes. The extracts were stored in the refrigerator for the synthesis of Ag nano-particles from AgNO3, and phytochemicals screening.

2.4 Qualitative Phytochemicals Screening of *Cucumis sativus* peel aqueous extract.

The techniques describes by Tiwari *et al.* [12] was used for the phytochemicals screening, in which the concentrated extracts of the pulverized *Cucumis sativus* peel aqueous extract was screened for the presence of alkaloids, tannins, saponins, glycosides, steroids, flavonoids and Anthraquinone.

2.4.1 Test for alkaloid

Cucumis sativus peel aqueous extract (0.5 ml) was added to 5 cm3 1% aqueous HCl and was stirred. A drops of Dragren droffs reagent (potassium bismuth iodide solution) was added, and 1 cm3 portion of the solution formed was mixed with wagners reagent (solution of iodine in potassium iodide). A white precipitate formed indicated the presence of Alkaloids [13].

2.4.2 Test for tannins

Cucumis sativus peel aqueous extract (0.5 ml) was boiled in 20 cm^3 of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and brownish green color indicated the present of tannin [14].

2.4.3 Test for saponins

Cucumis sativus peel aqueous extract (0.5 ml) was boiled in 20 cm3 of distilled water in a water bath and filtered, and 10cm3 of the filtrate was mixed with 5 cm3 of distilled water and shaken vigorously until stable persistent froth formed. The frothed was mixed with 3 drops of olive oil and shaken vigorously and an emulsion observed indicted the present of saponnins [14].

2.4.4 Test for flavonoids

The method described by Kumar *et al.* [15] was adopted in this research. 5 cm3 of dilute ammonia solution was added to a portion of the filtrate obtained from the extract above and concentrated H_2S0_4 was added. A yellow colouration observed which disappeared on standing indicated the presence of flavonoids.

2.4.5 Test for terpenoids

Salkowski method was adopted in this research. To 5 cm^3 of the oil, 2 cm^3 of chloroform was added and 3 cm^3 concentrated H_2S0_4 was carefully added and a reddish brown coloration at the interface of the layer formed indicated the presence of terpenoids.

2.4.6 Test for cardiac glycosides

According to Keller-Killani test/method adopted[16] in which 5 cm3 of oil was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution under layed with 1 ml of concentrated sulphuric acid. A brown ring at the interface observed indicates the presence of deoxysugar, characteristic of cardenolides.

2.5 Synthesis of Ag Nanoparticles using aqueous peel extract of *Cucumis sativus*

AgNO powder was dissolved in distilled water to prepare 10 mM AgNO3 stock solution from which a series of 1 mM, 3 mM, and 5 mM AgNO3 solutions were prepared. The AgNO solutions were mixed with the aqueous extract of *Cucumis sativus* peel at a ratio of 1 : 1 (v/v) to a volume of 50 mL in a flask each separately . The flasks were wrapped with an aluminum foil and was then heated in a water bath at 60^oC for 5 hours. Furthermore, the mixture was stored in the refrigerator for phytochemicals, characterization using UV-Vis spectrophotometer and transmission electron microscopy (TEM).

2.6 Confirmation of AgNO particle formation

The color change in the reaction mixture (metal ion solution + aqueous peel extract of *Cucumis sativus* was recorded through visual observation.

2.7. Characterization of Ag Nano-particles.

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-1800 (Shimadzu) at the wavelength of 200– 800 nm. Transmission Electron Microscope (TEM), operating at 120 V with a voltage of 15 kV, was used to analyze the morphology and size of Ag nano-particles. For TEM measurements, the *Cucumis sativus* silver nano particles (CP-AgNO3) and was dispersed on a copper grid and dried at room temperature. The particle sizes of the Ag nano-particles were measured using Image J software. The histogram of the size distribution was established by Origin software.

III. RESULTS AND DISCUSSION

3.1 Formation of Silver nana particles (AgNo) from *Cucumis sativus* Peel Aqueous Extract

The *Cucumis sativus* peel aqueous extract was used to synthesised silver nanoparticles (AgNPs), the extract act as reducing, as well as stabilizing agents in the aqueous medium. Following the addition of *Cucumis sativus* peel extract to 1 mM, 3 mM and 5 mM AgNO3 solution at 1.1 (v/v), the colour changed after 1 h from yellowish to reddish brown and became dark brown after 5 h (figure 1a-c). The change in colour indicated the formation of AgNPs in the mixed solution and the capping between Ag+ and *Cucumis sativus peel* aqueous extract has taken place [17]. The colourless silver ions was dissociated in AgNPs which was brown in colour. And the Excitation of surface plasmon vibrations in AgNPs is associated with the variation in colour changes observed. Similarly, the concentration of $AgNO_3$ solution at a fixed concentration of 5 mM with increases in *Cucumis sativus* peel extract at ratio 1:1 (v/v) were also conducted. The concentration of *Cucumis sativus* peel aqueous extract at (2, 5 and 20 % (w/v) mixed with 5 mM $AgNO_3$ solution after 24 h, the solution mixture containing 20 % (w/v) *Cucumis sativus* peel aqueous extract turned into darker brown when compared with the solution containing 2 % (w/v) and 5 % (w/v) of the extract. Intensity of absorption increased as the concentrations of *Cucumis sativus* peel aqueous extract increased (Fig. 2a-c) [18]. This may be associated with biological mediating nanoparticles present in the solution which increased AgNPs reductive process. As such intensity of the colour of solution was produced . Maximum absorption was recorded at 449 nm when the higher concentration of the *Cucumis sativus* extract was used.

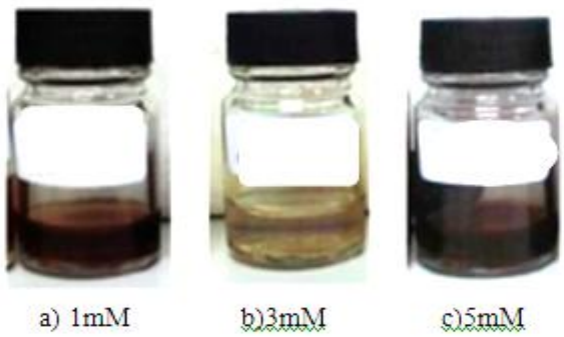


Figure 1a-c: Colour change indicating AgNPs formation at (a) 0 h, (b) 24 h, and (c) 48 h after missing with cucumber peel aqueous extract at 1:1 (v/v) with 1 mM, 3mM and 5mM AgNO3 solution

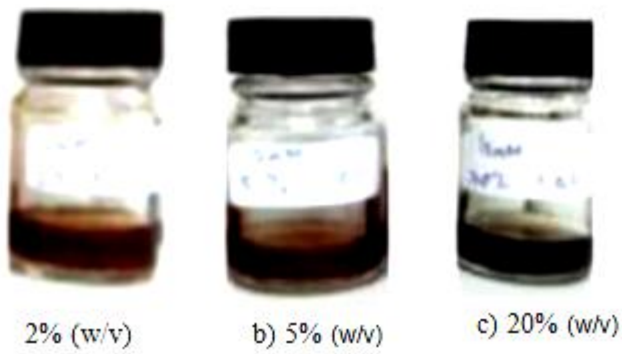
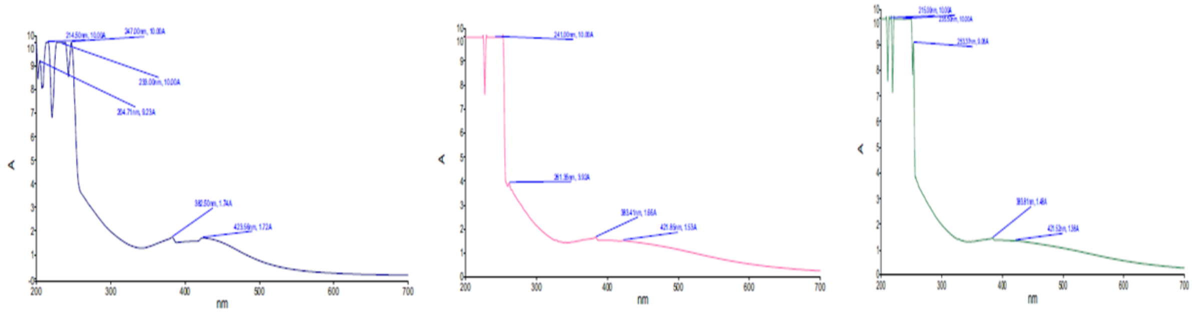


Figure 2a-c: colour changes at different concentration of *Cucumis sativus* peel extract after 24 h at a 5mM AgNO3 s olution at a ratio of 1:1 (V/V) .

3.2 UV-Vis spectroscopy of *Cucumis sativus* peel silver nanoparticles (CP-AgNPs)

The brown color of AgNPs obtained is associated with the vibration of free electrons of the metallic silver that are in resonance with the light wave. This explains the origin of the surface plasmon resonance (SPR) absorption often observed with metallic nanoparticles, this was verified using UV-Vis spectroscopy in order to support the visual observation made in confirming the AgNPs formation as indicated in Figure 3. The synthesized AgNPs obtained from *Cucumis sativus* peel aqueous extract exhibited distinctive UV-Vis absorption bands with maximum absorbances at 423.56 nm, 421.85 nm and 421.52 nm for the nanoparticles obtained at three (3) different concentration of 2,5 and 20%(w/v) of the *Cucumis sativus* peel extract respectively. The observed UV-Vis bands are due to the SPR absorption and confirm the p resence of AgNPs [19]. Additionally, there were significant differences in the maximum absorption wavelengths between the AgNPs from the three concentration. This could be due to the changes in particle size. This is similar to the report of Masrina *et al* [20] which used *Gynuraprocumbens* aqueous extracts for the synthesis of AgNPs.

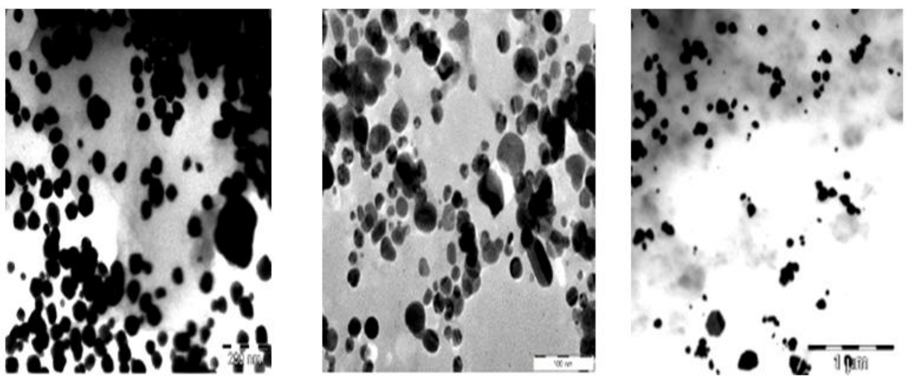


UV-VIS of 2% cucumber peel nano particles   UV-VIS of 5% cucumber peel nano particles   UV-VIS of 20% cucumber peel nano particles

Figure 3 : Absorption spectra at different concentration of *Cucumis sativus* peel extract after 24 h at a 5mM AgNO3 solution at a ratio of 1:1 (V/V)

3.3 Analysis of TEM images

The microscopic observation provides information for the synthesized nano-particles (AgNPs) in most biosynthetic medium. As shown in (Figure 4) a typical TEM images of silver nanoparticles synthesized from aqueous peel extract of *Cucumis sativus* (CP-AgNPs). The micrographs show the presence of individual nanoparticles which are spherical in shape and no particle aggregates; this is a typical characteristic features of AgNPs [21] . The particle sizes and distribution of CP-AgNPs at different concentration of 2, 5 and 20%(w/v) of the *Cucumis sativus* peel extract were estimated under TEM visualization using Image J software, and were found to be about 45 nm (with polydispersity of 32.1%), 115 nm (with polydispersity of 10.8%) and 47 nm (with polydispersity of 46.0%), respectively. This confirms the nano-particles nature of the synthesized CP-AgNPs and is in-line with the result obtained from the UV-Vis spectroscopy, for the successful formation of colloidal particles from silver ions in the presence of aqueous peel extracts of the *Cucumis sativus*. Also all the Image J graphs described a Gaussian distribution peak at around the average sizes estimated for each nanoparticle formulation (Figure 4), which shows acceptable particle size distribution as observed with polydispersity values below 50% [22].



TEM of 2% cucumberpeel nano particles   TEM of 5% cucumberpeel nano particles   TEM of 20% cucumberpeel nano particles

Figure 4: AgNPs shape and size distribution: TEM micrographs and Image J graphs of AgNPs obtained using *Cucumis sativus* peel extract at 2,5 and 20% w/v

3.4 Qualitative Phytochemicals Screening of AgNPs Obtained Using *Cucumis sativus* Peel Extract

The phytochemicals screening of AgNPs synthesized Using *Cucumis sativus* Peel Extract shows the presence of tannins, saponnins, flavonoids, cardiac glycosides, and steroids in an appreciable amount while alkaloids, terpenoids and anthraquinones were found to be absent (Table 1). Tannins were known to show good medicinal properties and have exhibited good physiological activity. And possessed strong anti-microbial effects [23]. Saponnins served as expectorant and emulsifying agents as well it has good antifungal activity [23] .While Flavonoids and glycosides are used for management of many diseases, hence their usage in herbal medicine [24]. The AgNPs obtained using *Cucumis sativus* peel extract contains steroids. This indicated that the presence of steroids proved the important and the emphasis of it pharmaceutical role in the development of sex hormone and other reproductive related compounds [25]. Anthraquinones is extensively used to prevent plant from many diseases and possess strong antimicrobial activity [26].

Table 1: Phytochemicals Contents of Synthesized AgNPs of Cucumber Peel Extrac

S/N	Phytochemicals	Inference
1	Alkaloids	-
2	Anthraquinones	++
3	Cardiac glycosides	++
4	Flavonoids	+++
5	Saponins	++
6	Steroids	+
7	Tannins	++
8	Terpenoids	+

IV. CONCLUSION

In this study, the eco-friendly, inexpensive, facile and fast green synthesis of AgNPs using aqueous peel extracts of *Cucumis sativus* which is used as a waste after domestic used. The colour changes from colourless to reddish brown observed is the distinguished properties of of silver nanoparticles due to SPR phenomenon. The UV-Vis spectroscopy confirmed the formation of silver nanoparticles with absorption bands at 434 nm, 489 nm and 522 nm for nanoparticles synthesized at three (3) different concentration 2,5 and 20%(w/v) of the peel aqueous extract. The TEM revealed spherical shape and no particle aggregates obtained,this served as a typical characteristic features of AgNPs .The synthesized *Cucumis sativus* peel nano particle (CP-AgNPs) shows the present of phytochemicals such as, tannins, saponins, flavonoids, glycosides , steroids and Terpenoids which are responsible for the reducing and capping ability of the biologically synthesized of silver nanoparticles from *Cucumis sativus* peel.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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