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Phytochemical and Synthesis of Silver Nanoparticles Using *Citrus Aurantifolia* Leaf Extract

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Abstract: *This studdy investigates synthsis and antimicrobial activity of silver nanoparticles using citrus aurantifolia leaf extract to achieve, the fresh leaf of citrus aurantifolia was collected, authenticated, prepared, extracted with de-ionzed water and concentrated using standard procedures. Four different concentrations of plant extract were prepared in the ratio of (1:1, 2:1, 3:1, 4:1) by increasing the concentration of plant extract in the solution mixture. 0.17% of 1mM AgNO3 metal ion was added to the prepared plant extract. Then the bio-reduced aqueous component solution turned colloidal and different in colour, which suggested the formation of Citrus auratifolia leaf extract silver nanoparticles The nano products formed were coded (As, Bs, Cs, Ds).However, a control solution was made by 10 mL of citrus auratifolia leaf extract (solution) without silver nitrate solution in which no colour change was observed. Citrus aurantifolia leaf extract reveals the presences of some phytochemicals such alkaloids, flavonoids, saponins and tannins respectively and it could be served as a potential agent of synthesizing silver nanoparticles.*

Keywords: *Citrus Aurantifolia, Silver Nanoparticles, Phytochemical.*

INTRODUCTION

Nano is a metric measure of one billionth of a meter and covers a width of 10 atoms. In terms of comparison with real objects, an example that hair is 150,000 nanometers may be given. The rapidly developing nanotechnology is the inter-disciplinary research and development field of biology, chemistry, physics, food, medicine, electronics, aerospace, medicine, etc., which examines the design, manufacture, assembly, characterization of materials that are smaller than 100 nanometers in scale, as well as the application of miniature functional systems derived from these materials. It represents the whole of development activities. As far the nanobiotechnology, on the other hand, it is the result of a combination of biotechnology and nanotechnology branches with a common combined functioning (Pearce, 2012). The reason for the intense interest of scientists nowadays in nanoparticles is that nanoparticles can exhibit different properties and functions than normal bulk materials. The most important factor that enables production of nanostructures in desired size, shape and properties and provides their usage in various fields is that the effects of classical physics are reduced and the quantum physics becomes active. Other reasons for the different behavior of nanoparticles in physical, chemical, optical, electrical and magnetic behavior include the limitations of load carriers, size dependent electronic structures, increased surface / volume ratio, and other factors incurred by the unique properties of atoms (Shah *et al*., 2015).

The process of removing toxic and waste metals in the environment includes microorganisms, plants and other biological structures; achieved by means of oxidation, reduction or catalysis of metals with metallic nanoparticles. Metallic nanoparticles produced by biological methods; are used in the biomedical field for purposes such as protection from harmful microorganisms, bio-imaging, drug transport, cancer treatment, medical diagnosis and sensor construction because of their unique properties such as being insulator, optics, antimicrobial, antioxidant, anti-metastasis, biocompatibility, stability and manipulability (Schrofel *et al*., 2014; Singh *et al*., 2016).

Citrus aurantifolia (christm) swingle (lime) belong to the family rutaceae. It is a dense and irregularly branched tree. The stem is spreading and woody, brown in color, with short stiff spines on twigs. The leaves are acute, entire elliptic, oblong-ovate, dark green above, pale-green blow, altanate with narrowly winged leaf petioles. Flowers are white and stand from lea corners. The fruit is round, greenish-yellow with thin skin, juicy, fragrant and very acidic. The seeds are small, avoid and pale (Aliyu, 2006).

Citrus aurantifolia in its natural state is widely used in West Africa, particularly in Nigeria where it is employed in herbal medicine to treat several illnesses. It forms an essential ingredient in the preparation of most herbal concoctions (Aibinu *et al*., 2007)

This research work was designed to carry out phytochemical and synthesis of silver nano particles using citrus aurantifolia leaf extract will help provide scientific baseline information on phytochemical and plant based silver nanoparticles.

MATERIALS AND METHODS

Sample collection and Extraction

Citrus aurantifolia L. (Lime) leaf: Fresh leaf of *Citrus aurantifolia*(CA) L. was collected at Mashidimami garden along Damboa road, Maiduguri. The plant part was authenticated by a botanist in the Department of Biological Sciences, University of Maiduguri, Borno state and washed several times with water to remove the dust particles and then air dried to remove the residual moisture and pulverized into powder. Then plant extract was prepared by mixing 1% of plant extract with deionized water in a 250ml of conical flask. Then the solution was incubated for 30 min. and subsequently subjected to centrifuge for 30 min. at room temperature with 5000 rpm. The supernatant was separated and filtered with (mm filter paper pore size) filter paper with the help of vaccume filter. Then the solution was used for the reduction of silver ions Ag^{+}) to silver nanoparticles (Ag^{o}).

Synthesis of Silver Nanoparticles

Four concentrations of plant extract were prepared (20, 40, 60, 80 mL) in 20 mL of 1 mM silver nitrate in the ratio of (1:1, 2:1, 3:1, 4:1) by increasing the concentration of plant extract in the solution mixture. 0.17% of 1mM $AgNO_3$ metal ion was added to the prepared plant extract. Then the bio-reduced aqueous component solution turned colloidal and different in colour, which suggested the formation of *Citrus auratifolia* leaf extract silver nanoparticles however, a control solution was made by 10 mL of *citrus* leaf extract (solution) without silver nitrate solution.

After incubation, the solution was centrifuged at 12000 rpm for 4 minutes, and the obtained precipitate mass i.e., *Citrus auratifolia* leaf extract silver nanoparticles was washed three times with distilled water and centrifuged at 12,000 rpm for 3min.The mass was collected and oven dried at 42°C. Thereafter *Citrus auratifolia* leaf extract silver nanoparticles so obtained were scraped, coded (As, Bs, Cs, Ds) and kept for further analysis.

Phytochemicals Screening

The plant was dried under shade and pulverized into power. Two hundred grams (200g) of powered plant material was soaked in stopped container with methanol for three day with frequent agitation. The extract was filtered, concentrated. The methanol extract was screen for its phytochemical using standard procedures described by (Brain and Tuner, 1975; Markham, 1982; Sofowora, 1993; Trease and Evans, 2002).

Table 1: The Result of Synthesis of Silver Nanoparticles Using Citrus Leaf Extract

S/NO	Sample	Ratio(v/v)	Colour after 24 hrs	*Citrus a.* leaf extract silver nanoparticle
1	A	1:1	Tan	As
2	B	2:1	Latte	Bs
3	C	3:1	Linen	Cs
4	D	4:1	Off white	Ds
5	E	-	Yellow	-

Key:
A, B, C, D = *Citrus aurantifoloia* leaf extract solution + $AgNO_3$
E= *Citrus aurantifoloia* leaf extract solution
(As, Bs, Cs, Ds) = *Citrus a.* leaf extract silver nanoparticles

Table 2: The Result of Phytochemical screening of citrus aurantifolia leaf extract

TEST	RESULT
Alkaloids	+
Anthroquinones	-
Cardiac-glycosides	-
Flavonoids	+
Saponins	+
Tannins	+
Terpenoids	-

Key: + = Present

- = Absent

Discussion

The synthesis of the silver nanoparticles was confirmed by the characteristic colour change from greenish to tan, latte, linen and off white respectively, for the ratios of 1:1, 1:2, 1:3 1:4 of *Citrus a.* leaf extract solution together with AgNO3 as shown in the (Table 1). The nano products formed were coded (As, Bs, Cs, Ds). This result is in consonance with the findings of Sivakumar and Vidyasagar (2014) reported that during the synthesis of silver nanoparticles using Annona reticulata the color of the reaction mixture after 20 min, at room temperature, changed to dark brown, indicating the formation of AgNPs.

The result of phytochemical screening of methanolic leaf extract of *citrus aurantifolia* revealed the presence of alkaloids, flavonoids, saponins and tannins respectively, among the metabolites, alkaloid have been reported to have analgesic properties this shows that the plant part could be considered as a potential pain reducing agent. Moreover, tannins Tannins are polyphenols that are obtained from various parts of different plants (Gajendiran and Mahadevan,1990). In addition to its use in leather processing industries, tannins have shown potential antiviral and antibacterial. (Lin *et al*., 2004; Akiyama *et al*, 2001; Funatogawa *et al*., 2004; Yangand Kun-Ying, 2000). But the metabolites such as terpenoids, cardiac-glycosides and anthroquinones were not found in the extract.

Conclusion

In conclusion, this study reveals the results of phytochemical and synthesis of *Citrus aurantifolia* leaf extract silver nanoparticles. The result of this study shows that *Citrus aurantifolia* leaf extract contain some phytochemicals such alkaloids, flavonoids, saponins and tannins respectively and it could be served as a potential agent of synthesizing silver nanoparticles.

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