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Research Article

# *In-vitro* Anti-hypertensive, Anti-oxidant and Anti-fungal Properties of Green Synthesized Gold Nanoparticles from I*cacina Trichantha* Leaf Aqueous Extract

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## Abstract

Nanotechnology is one of the leading innovations in this dispensation; this research describes the green synthesis of gold nanoparticles from *Icacina trichantha,* and some of its biomedical applications. The extract of *Icacina trichantha* served as the reducing/capping agents while the gold chloride solution served as the precursor, the reaction resulted in colloidal suspension formation after photo-activation. The colloidal suspension was characterized using UV-Vis spectroscopy, FTIR, EDX and SEM. The UV-Vis spectrum of the AuNPs displayed strong peak at 562 nm. The FTIR showed prominent peaks at 3425.69, 2928.04, 2362.88, 1763.00, 1635.69, 1384.94 and 1089.82 $cm^{-1}$, these are attributed to the involvement of proteins in the AuNPs bio-fabrication and capping. Gold was the most occurring metal noted in the EDX analysis while the SEM micrograph of the nanoparticles showed sizes between 23.34 – 79.69nm.

The AuNPs showed antifungal activity of 64.1%, 75.9%, 70.6%, 72.6% and 77.1% against *Fusarium solani, Fusarium poae, Aspergillus niger, Aspergillus flavus* and *Penicillium avenatum* respectively at 150 µl /ml. The AuNPs showed significant antioxidant properties of 78.11%, 79.43% and 81.56% at 50, 100, 150 and 200µl/ml against DPPH. The AuNPs showed a significant decrease in angiotensin converting enzyme (ACE) inhibitory activities as the concentration increased. The AuNPs showed angiotensin converting enzyme inhibitory activities of 44.94 ± 1.094, 40.14 ± 0.3604, 37.25 ± 0.06860 and 36.76 ± 0.03091% at concentration 50, 100, 150 and 200 µg/ml respectively. Conclusively, the AuNPs could be applied in fumigants production, and formulation of agents against oxidative stress. The nanoparticles also displayed anti-hypertensive activities and could be used as a therapeutic agent in the control of blood pressure.

**Keywords:** Gold Nanoparticles; Antioxidant; Antifungal; Anti-Hypertensive

## Introduction

The dawn of the 4th industrial revolution has given birth to unprecedented scientific advancements like artificial intelligence, virtual reality, and nanotechnology; these feats of innovation have paved ways for human kind more than ever before. Nanotechnology remains one of the most versatile of them all as it cut across all fields illuminating the possibilities in manipulation of matter at atomic levels [1]. Nanotechnology has been employed in food biotechnology, therapeutic and biomedical areas [2]. Food packaging, drug delivery system and miniaturization of biosensors have never remained the same since the advent of nanotechnology [3]. The synthesis of nanoparticles, the key agent in nanotechnology, has been achieved in diverse ways, concisely, the chemical, mechanical and the green method. The most ecofriendly approach

in the synthesis of nanoparticles is through the green method and this involves the use of bioactive molecules in plants, animal tissues and microorganisms [3,4].

The use of medicinal plants bioactive molecules in the synthesis of nanoparticles have given birth to a plethora of biomedical breakthroughs. The plant richness in phytochemicals and macromolecules has made them inevitable to man's survival. The use of medicinal plants in the treatment and management of diseases is as old as creation, and in this dispensation, through nanotechnology, the richness of plants in biomolecules have been utilized in the bio-reduction of bulky chemical component to particle sizes thousand time smaller than a micron (i.e. $10^{-9}$) thus improving the reactivity, surface area and pathways [1]. Many medicinal plants like *Azadirachta indica* [5], Aloe vera leaf [6], Coriander leaf extract [7, 8], *Diopyros kaki, Magnolia Kobus* [9], and chloroplast of Trifolium leaves [1,10] have been used in the synthesis of gold nanoparticles. In this research work, *Icacina trichantha* was used in the green synthesis of gold nanoparticles and its in-vitro, antihypertensive, antioxidant and antifungal properties were evaluated. *Icacina trichantha* is a common medicinal shrub which belongs to the Icacinaccae family.

In West Africa especially Nigeria, it is locally called Ewe gbegbe in Yoruba dialect [11] and its richness in pharmacological properties make it versatile and highly useful in folk medicine [12,13]. *Icacina trichantha* has been reported to have hepatoprotective activity [14], antioxidant properties [15,16] anti-inlamatory properties [1] and antimicrobial properties against pathogenic bacteria [13,17]. *Icacina trichantha* has been reported to contain a great deal of phytochemicals like alkaloids, phenols, saponins and tannins [18]. Also the plant contains organic acids like crucic acids, oleic acids and stearolic acids [1,13]. The medicinal and pharmacological feats of *Icacina trichantha* prompted this research.

## Materials and Methods

### Synthesis and characterization of gold nanoparticles

The gold nanoparticles (AuNPs) were bio fabricated from the aqueous extract of *Icacina trichantha* leaf. The plant aqueous extract was obtained through hot water (60°C) extraction of dried leaf powder (1 g in 100 ml of distilled water) and then exactly 0.5 ml of the aqueous leaf extract was reacted with 10 ml of 1mM $HAuCl_3$ under ambient condition. The gold nanoparticles were characterized using UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), Energy dispersive X-ray (EDX) analysis and scanning electron microscopy (SEM).

### *In-vitro* antihypertensive assay

### Angiotensin I-Converting-Enzyme (ACE) inhibitory activity

Angiotensin I-Converting-Enzyme (ACE) Inhibitory activity was assayed by measuring the release of Hippuric acid (HA) from the substrate Hippuryl-L-Histidyl-L-Leucine (HHL) according to Jimsheena and Gowda [19]. The assay mixture contained 0.125 ml of a 0.05M Sodium bromate buffer(pH8.2) containing 0.3M Nacl, 0.05 ml of 5M mHHL and 0.025ml of ACE (2.5MU) which was pre-incubated with different nanoparticles concentrations of 50, 100, 150 and 200 µg/ml. The reaction was stopped after incubation at 37°C for 30 minutes by the addition of 0.2ml of 1MHCl pyridine (0.4 ml) was added followed by 0.2ml of Benzene Sulphonyl Chloride (BSC), the order of addition of reagents is critical, and mixed by inversion of 1 minute and cooled on ice. Absorbance was measured at 410nm in amicrotiter plate. The degree of ACE inhibition (%) was calculated with the formula:

$$\text{ACE inhibition } \% = \frac{A1-A2}{A1-A3} \times 100$$

A1 = Absorbance of the ACE solution without an inhibitor (*Icacina trichantha*)

A2 = Absorbance of the tested sample of *Icacina trichantha*

A3 = Absorbance of HHL solution (a buffer was added instead of ACE solution and sample)

### Antioxidant activities of biosynthesized gold nanoparticles

### 2, 2-diphenyl-1-picryhydrazyl (DPPH) scavenging Potentials

The antioxidant activity of the nanoparticles was measured by using the modified DPPH method as reported by [20]. 0.04g of DPPH was dissolved into 1000ml of methanol and the optical density of the solution was taken at 517nm. To determine the antioxidant activity of the nanoparticles, different concentrations of the synthesized gold nanoparticles (50, 100, 150 and 200µl/ml) were reacted with 4ml of the DPPH, the nanoparticles were allowed to scavenge for DPPH in the dark for 30 minutes and then the absorbance was measured at 517 nm in UV-visible spectrophotometer. The scavenging percentage was calculated using the formula below:

$$\text{DPPH scavenging (\%)} = \frac{\text{Absorbance control} - \text{Absorbance of sample}}{\text{Absorbance control}} X\ 100$$

### Antifungal activity

The mycelial inhibitory activities of the gold nanoparticles were evaluated using Mycelial Inhibition Method [20] through the incorporation of 50 µl/ml of the synthesized gold nanoparticles into potato dextrose agar plates, which were then inoculated with agar plug (8 mm) of 48-h old pure cultures of *Aspergillus niger, Aspergillus flavus*, *Fusarium poae, Fusarium solani* and *Penicillium avenatum*. In the control experiments, fungal plugs were inoculated on PDA plates without the incorporation of the gold nanoparticles. All the plates were incubated at 28 ± 2$^0$C for 72 hours. The diameters of fungal growths in all the plates were measured and used to determine the percentage growth inhibitions as follows:

$$\frac{\text{Dcontrol} - \text{Dtest}}{\text{Dcontrol}} X\ 1\,00\%$$

Where D is the diameter of fungal growth on the PDA plates.

### Statistical analysis

The statistical analysis of data was done using Statistical Package for Social Sciences (SPSS) 21.1. The data were expressed as mean ± SEM and analyzed using One-way Analysis of Variance (ANOVA). The difference between concentrations of control and test groups was determined using Duncan test and considered at p value < 0.05.

## Results and Discussion

### Biofabrication and characterization of the gold nanoparticles

The aqueous extract of *Icacina trichantha* leaf facilitated the bio-fabrication of the gold nanoparticles as shown in figure 1. The bio-fabricated gold nanoparticles stabilized within 15 minutes at photoactivation. The appearance of deep blue coloration established the formation of a new substance and according to Oladipo., *et al*. [20] variation in the color of gold nanoparticles such as pink, blue, golden brown, and purple [6,9,21] has been related to the composition of bioactive molecules responsible for the bio-fabrication of the gold nanoparticles which lend credence to the deep blue color observed at photo-activation when the reaction stabilized. Furthermore, the change in coloration is attributed to the excitation of surface plasmon vibration, which is an indication that $Au^{3+}$ ions is reduced to $Au^0$ ions at different time interval [20,22].

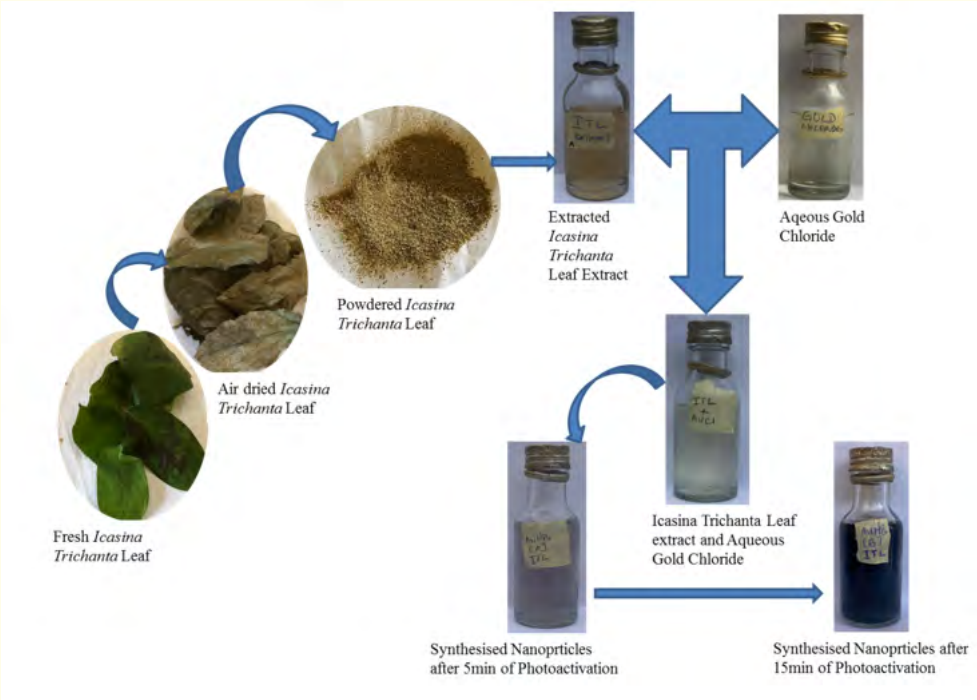


**Figure 1:** Sample preparation and green synthesis of gold nanoparticles from *Icacina trichantha* leaf aqueous extract.

The UV-vis spectrum of the gold nanoparticles displayed maximum absorbance at the wavelength of 562 nm as shown in figure 2 and this wavelength is within the range of 510 to 614nm respectively reported by Mishra., *et al*. [23] and Oladipo., *et al*. [20].

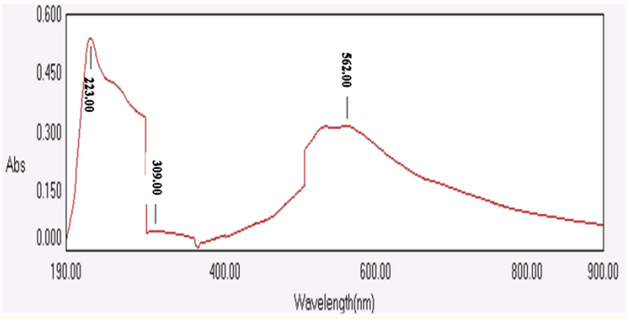


**Figure 2:** Ultraviolet visible spectrum of the biosynthesized gold nanoparticlesfrom *Icacina trichantha* leaf extract.

The gold nanoparticles FTIR spectrum showed prominent broad peaks at 3425.69, 2928.04, 2362.88, 1763.00, 1635.69, 1384.94 and 1089.82 $cm^{-1}$ (Figure 3). The vibrational stretch around 3425 $cm^{-1}$ is ascribe to N-H amines/amides and O-H group. The peak at 2928.04 is assigned to C-H stretch of the alkyl group, the peak around 2362.88 $cm^{-1}$ correspond to C≡C from alkyne and the peak at 1635.69 to 1384.94 $cm^{-1}$ portended the conjugation effects of C = O stretching of N-H binding of protein, carbonyl groups or esters [24]. All these vibrational stretch are establishing the presence and bioreducing properties of macromolecules like carbonyl compounds macromolecules, proteins and phenolic compounds found in the *Icacina trichantha* leaf aqueous which was confirmed by Chun-Tao., *et al*. [11] that alkaloids, phenols, saponins and tannins are present. The Energy Dispersive X-ray (EDX) analysis (Figure 4) showed that gold most occurring metal noted. The scanning electron microscope (Figure 5) micrograph showed that the gold nanoparticles is agglomerated and elongated within the size of 23.34 – 79.69nm, this is in support with the report of Shankar., *et al*. [5] and Chun-Tao., *et al*. [11].

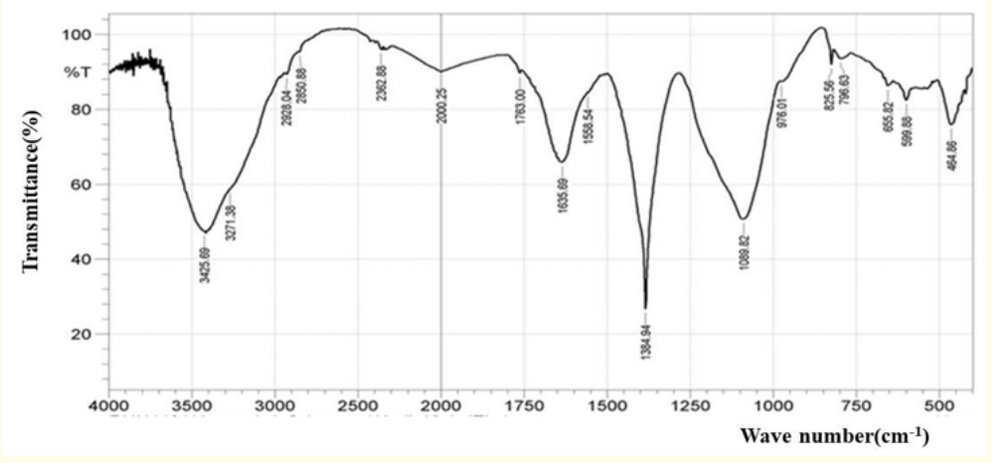


**Figure 3:** Fourier transform infrared spectrum of the synthesized gold nanoparticles from *Icacina trichantha* leaf extract.

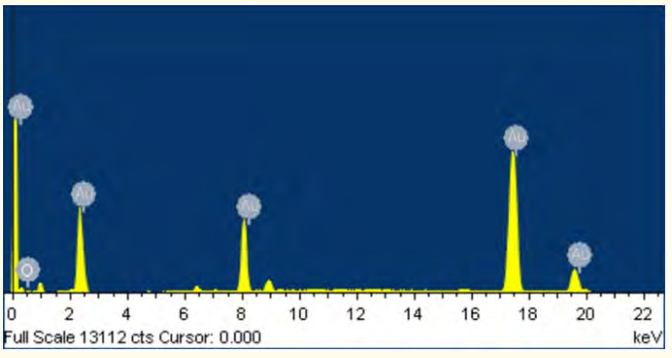


**Figure 4:** Energy dispersive x-ray spectrum of the phytosynthesized gold nanoparticles from *Icacina trichantha* leaf extract.

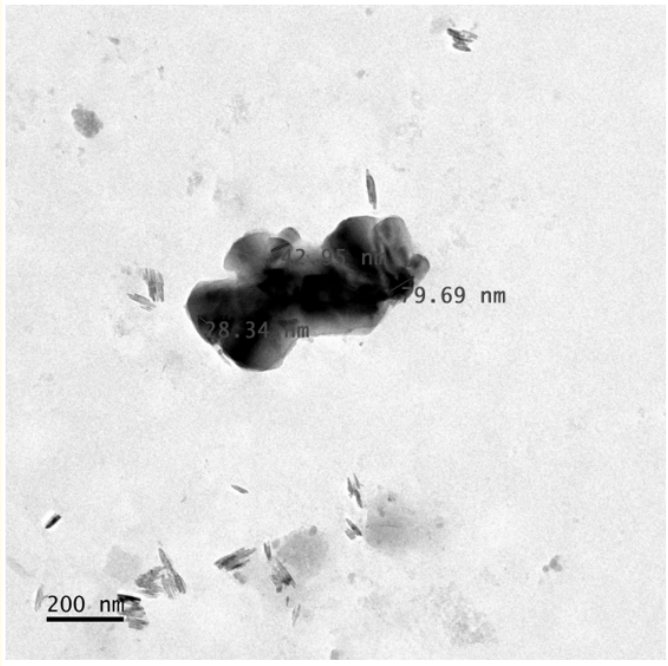


**Figure 5:** Scanning electron microscope micrograph of the synthesized nanoparticle from *Icacina trichantha* leaf extract.

Hypertension, a worldwide illness, is a major factor in cardiovascular diseases that affects a large population of adults. One of the most effective medications for the treatment of hypertension is angiotensin converting enzyme inhibitors. Meanwhile, medicinal plants have been used for treating illnesses. Therefore, they can be important resources to develop new drugs [25]. As observed by Sharifi., *et al.* [26] in their usage of medicinal plant in the inhibition of angiotensin converting enzyme, it was observed that plants which showed inhibition activity more than 50% are said to be effective in the inhibition of angiotensin converting enzyme at 330µg/ml. From this research however, ACE inhibition activities of the synthesized gold nanoparticle (Figure 6) was carried out according to Jimsheena and Gowda [19] and it was observed that there was high ACE inhibition activities at the concentration 50 µg/ml compared to the standard which inhibited ACE activities at 200 µg/ml with the result 44.94 ± 1.094 and 85.90± 2.8560% respectively.

The results obtained implies that the nanoparticle was effective at a lower concentrations in the ACE inhibitory activities, in that, less dosage of the nanoparticles would be administered to hypertensive patients as against the usage of standard at higher concentration. This is in tune with the observation of Hassani., *et al.* [27] in their research to determine whether magnesium orotate nanoparticles (MgOrGANPs) was effective in ACE inhibitory activity compared with magnesium orotate (MgOr), and it was observed that MgOrGANPs were demonstrated to have more potent ACE inhibition activity compared to MgOr at the concentration of 5 µg/ml [27].

The gold nanoparticles showed excellent DPPH radical scavenging properties against 2, 2- diphenyl-1-picryhydrazyl (DPPH) by 78.11, 79.43, 80.34 and 81.56% at 50, 100, 150 and 200 µl/ml (Table 1). The scavenging activities of green synthesized gold nanoparticles have been lent credence to by Arockiya., *et al*.

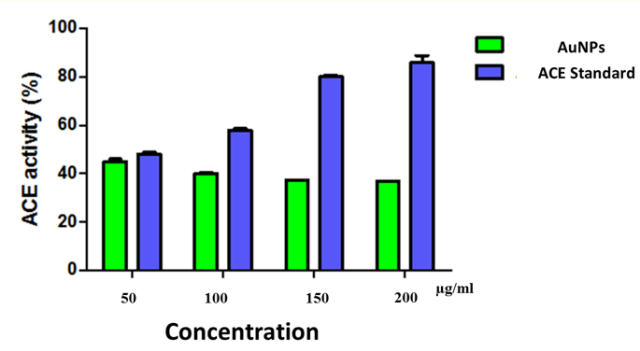


**Figure 6:** Histogram representation of the anti-hypertensive properties of synthesized gold nanoparticles from Icacina trichantha leaf.

Where:

T in Concentration D shows that there is a wide difference in the three values of Concentration D.

| Organisms | Antifungal activities of AuNPs (%) |
|---|---|
| *F. solani* | 64.1 |
| *F. poae* | 75.9 |
| *A. niger* | 70.6 |
| *A. flavus* | 72.6 |
| *P. avenatum* | 77.1 |

**Table 2:** Antifungal activities of the synthesized gold nanoparticles.

*Fusarium poae, Aspergillus niger, Aspergillus flavus* and *Penicillium avenatum* respectively at 150 µl /ml as compared to the abundant mycelial growth of the control. It was be established that the gold nanoparticles exhibited tremendous antifungal properties and this is in correlation with the report of Oladipo., *et al*. [20] on gold nanoparticles mediated with *Datura stramonium*. The reports are establishing the potency of gold nanoparticles against the spore and strong cell wall of the toxigenic fungi. The secondary metabolites of toxigenic fungi called mycotoxins have wrecked lots of havoc in both stored grains and food products. The spores of these toxigenic fungi are ubiquitous which made them almost impossible to prevent especially when the condition is conducive for their growth. Fusarium and Aspergillus species are the largest mycotoxin-producing fungi of them all [29,30] and that was what encouraged the selection of the test organisms in this study. Many developing countries are suffering great losses in agriculture as a result of these molds infestation. The gold synthesized nanoparticles could provide a lasting solution with no side effect or pollution because evidently, the toxigenic fungi used in this study have proven susceptible to the *Icacina tricchantha* mediated gold nanoparticles [31].

[28] who reported that *Suaeda monoica* leaf extract mediated gold nanoparticles displayed DPPH radical scavenging activity of 43% at 1mg/ml. On the evidence of the result comparison from Arockiya., *et al*. [28] and the one from this study on DPPH radical scavenging properties of the *Icacina tricantha* mediated gold nanoparticles, it could be established that the latter gold nanoparticles had more potent antioxidant properties.

| Samples | DPPH Free Radical Scavenging (%) |
|---|---|
| Ascorbic acid | 70.19 |
| Concentration of AuNps (µl/ml) | |
| 50 | 78.11 |
| 100 | 79.43 |
| 150 | 80.34 |
| 200 | 81.56 |
| Extract | 65.31 |

**Table 1**: 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) activities of the synthesized nanoparticles from *Icacina trichantha* leaf extract.

The gold nanoparticles showed significant effect against the mycelial growth of five known toxigenic fungi which are *Fusarium solani, Fusarium poae, Aspergillus flavus, Aspergillus niger* and *Penicillium avenatum* which were isolated from stored grains whose market value have been reduced due to mold infestation. The gold nanoparticles showed antifungal activity of 64.1%, 75.9%, 70.6%, 72.6% and 77.1% (Table 2) against *Fusarium solani,*

## Conclusion and Recommendation

This study has therefore established the relevance of the *Icacina trichantha* leaf aqueous extract in nano-biotechnological applications, particularly in green synthesis of low cost, eco-friendly, safe, reliable and stable gold nanoparticles. The synthesis of the gold naoparticles showed significant antifungal and anti-hypertensive properties. It also showed excellent free radical scavenging activities when screened with 2, 2-diphenyl-1- picryl-hydrazyl (DPPH) which might be useful in preventing or slowing the progress of various oxidative stress related disease. The gold nanoparticles could be further researched and embedded in therapeutic agent to control blood pressure and oxidant accumulation in the body.

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