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Synthesis, Characterization and Antimicrobial Activities of Copper-Tea Leaves (*Camellia Sinensis*) Extract Nanoparticles.

B. T. Iorhuna[a,*], T. T. Awuhe[b], I. C. Azuaga[a], E Isaac[a], F. Shuaibu[a], B. Yohanna[a]

[a] *Chemical Sciences Department, Taraba State University, Jalingo, Taraba State, Nigeria.*
[b] *Science Department, Federal Polytechnic of Oil and Gas, Bonny Island, Rivers State, Nigeria.*

Abstract

Nanoparticles, NPs synthesis has gained attention recently due to their ease of preparation (especially green synthesis), availability of raw materials and usefulness. The green synthesis of copper nanoparticles (CuNPs) was done using Tea leaves extract-harvested from the Mambilla in Taraba State-Nigeria. The phytochemical analysis of the tea leaves extract was done and found to contain phenols, steroids and saponins which could have caused colour change, reduction of copper ions, capping and stabilisation of the synthesized CuNPs. The presence of NPs in the mixture was identified by the change in colour of the mixture, λ_{max} of the ultraviolet-visible spectrophotometry and spectra of the Fourier Transform Infrared, FTIR spectrophotometry on the mixture. Antimicrobial studies of the synthesized CuNPs on the bacterial (*Escherichia Coli*) showed effective toxic effects. This study showed that, Tea leaves extract is good for synthesizing copper nanoparticles that can be used as antimicrobial agents.

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1. Introduction

Diseases causing organisms like fungi, bacteria, and viruses which are resistant to many drugs have caused a lot of issues Worldwide. This could be due to the unprescribed use of antimicrobial drugs that have led to increasing cases of drug resistance, thus bringing down the effectiveness of these drugs when used for treatment. Therefore, new procedures such as the development of alternative antimicrobial drugs or modifying the existing drugs are being sorted with high interest [1].

Nanoparticles could occur naturally or they can be engineered. They are very small in size and their particles sizes vary between 1 - 100 nm. They are unique in their properties and are highly valuable. Nanoscaled particles, display better properties (such as; catalytic, magnetic, electrical, mechanical, optical, chemical, biological, medical, etc). Because these particles have high surface/volume ratio, they show higher reactivity, mobility, dissolution properties, and strength compare to other materials [2]. The design, synthesis and manipulation of nanoparticles structure is an upcoming and relevant field of research and technology [3]. Nanoparticles obtained from metals have found a very good number of applications in nature, they can be applied in many industries (including catalysis) and medicine including; drug delivery, cancer treatment, wastewater treatment and DNA analysis, as

*Corresponding author tel. no: +2347030479355
Email address: boniface.tersoo@tsuniversity.edu.ng (B. T. Iorhuna)

antibacterial agents, as biosensors, in solar power generation, and many others [4].

Many methods (including chemical, physical and biological) can be used in the synthesis of nanoparticles. Although chemical method of synthesis takes a shorter period of time to synthesize a large quantity of nanoparticles, this method uses capping agents to stabilize the nanoparticles, and most of these chemicals are toxic and can result to by-products that are non-ecofriendly. Because we are all concerned with the use of environmentally friendly synthetic approaches for nanoparticles synthesis, this leads to the developing interest in biological approaches which reduce the use of toxic chemicals as by-products [5]. We shall prepare nanoparticles by green synthesis of metallic nanoparticles using locally available materials as a cost-effective and environmentally benign alternative to chemical and physical methods. Copper nanoparticles (CuNPs) research has been of great interest in recent years due to its applications in various fields of endeavour.

Tea leaves and many other plants (contain amino acids, alkaloids, proteins, polysaccharides, phenolics, terpenoids, flavones, and other active biomolecules) play important role in nanoparticles synthesis, these plants' phytochemicals act as both reducing and capping agents [6]. Amongst the biomolecules, phenols and flavonoids have unique chemical properties and can reduce and wrap NPs. This could be because of the presence of hydroxyl and carboxyl functional groups that can bind to the metal [7].

Tea is a drink that is an infusion of processed leaves and flowers of one of the varieties of an evergreen shrub botanically called *Camellia sinensis*. Tea is a common beverage that is drunk by many. Tea Leaves (*Camellia Sinensis*) is rich in polyphenolic compounds, and has been reported as a natural source in the synthesis of nanoparticles [3]. Green Tea is mainly composed by epigallocatechin, epigallocatechin-3-gallate (EGCG), epicatechin and epicatechin-3-gallate which act as reducing and capping agents [8, 9]. The green tea has many medical benefits and the phytochemicals obtained from Tea leaves have been used previously in cosmetic and therapeutic applications. The phytochemicals are mainly catechins and caffeine which are water-soluble and can act as reducing agents of metal ions, leading to the formation of capped metallic nanoparticles [9].

2. Materials and Methods

2.1. Materials

$Cu(NO_3)_2$. $2.5H_2O$, NaOH and iron(III) chloride (LOBA^{AR} CHEMIE PVT Ltd), H_2SO_4 (JHD^{AR}), Ethanol and iodine solution (Fenxichun^{AR}), nutrients agar and acetic anhydrate (Qualikems^{AR}), deionized water, Tea Leaves (*Camellia Sinensis*), Sieve (Cole Parmer-typed sieve of 0.80 mm mesh), pH meter (Hanna Instrument H19024) , UV-visible (UV Apel 3000 UV USA) and FTIR spectrophotometers (Agilant Technologies MicroLab PC).

2.2. Methods

2.2.1. Harvest and Preparation of Tea Leaves Extract

Fresh Tea Leaves were harvested around the premises of Kakara Beverage of Sardauna Local Government Area of Taraba State of Nigeria. It was dried at room temperature and grounded into a powder form through sieve. The Tea Leaves extract was prepared by taking 10 g of grounded Tea Leaves and dissolving it in a 500 mL beaker with the addition of 200 mL of deionized water and stirring for 25 minutes, Figure 1. The mixture was left to stand in a cupboard for 2 hours at room temperature. The extraction was done by filtration using Whatman filter paper [10].

2.2.2. Phytochemical Analysis of Tea Leaves Extract

Test for Steroids: this was done using the general procedure; 5 mL of chloroform was added to 1 mL of the Tea Leaves extract, then 5 mL of concentrated H_2SO_4 was then slowly added. The upper layer turned red and the acid layer turned yellow-green which showed the presence of steroids [11].

Test for Carbohydrates: to the fresh Tea Leaves Extract were added a few drops of iodine solution, and no reaction was observed indicating the absence of carbohydrates [10, 11].

Test for Flavonoids: these are tested according to this method; to the 4 mL of the Tea Leaves Extract were added 1 mL of a 2 N $NaOH_{(aq)}$, no reaction was observed (no yellow colour formation) indicating the absence of flavonoids [10, 11].

Test for Saponins: a test-tube containing 4 mL of aqueous extract of Tea Leaves was vigorously shaken, and a 1 cm foam layer was formed which indicated the presence of saponins [11].

Test for Phenols: to a 2.5 mL of ethanol was added equal volume of Tea Leaves extract and a few drops of iron(III) chloride. A yellow-greenish precipitate was formed indicating the presence of phenols [10].

2.2.3. Synthesis of Copper Nanoparticles, CuNPs

Nanoparticles derived from the Tea Leaves extract were synthesized using the methodology according to a previous study with some modifications [10].

2.2.4. Characterization of the Tea Leaves Extract and CuNPs

The Tea Leaves Extract and CuNPs functional (chromophore) groups were characterized by UV-visible absorption and Fourier-Transform Infrared (FTIR) spectrophotometry. In addition, the formation of the CuNPs was done with continuous observation of the change in colour of the mixture [12].

2.2.5. Antibacterial Activity of the CuNPs

Agar disc-diffusion method was used to determine the antibacterial activity of our prepared CuNPs against *Escherichia coli*, a gram-negative bacteria. The microbial strain was obtained from Federal Medical Center Jalingo, Taraba State, and

(a) (b) (c)

Figure 1: (a) Fresh Tea Leaves, (b) Dried Tea Leaves and (c) Grounded Tea Leaves

was cultured in a nutrient broth for 24 hours to obtain a growth phase of the strain bacteria. This actively growing bacterial culture was inoculated/spread into the Muller Hinton Agar (MHA). The CuNPs solutions were prepared at varying concentrations; 0.031, 0.063, 0.125, 0.250 and 0.500 mg/mL (dissolved in Dimethyl Sulfoxide, DMSO). The Whatman filter paper disks were punched at 6 mm diameter and spread through with the dissolved CuNPs and then placed to the MHA surface. Streptomycin disc was used as a positive control while DMSO was taken as the negative control. The plates were incubated at 37°C for 34 and 48 hours. The effect of synthesized CuNPs on the bacterial was evaluated in terms of zones of inhibition measured, and recorded in millimeters using a ruler [10, 12].

3. Results and Discussion

3.1. Phytochemical Analysis of the Tea Leaves Extract

The phytochemical analysis of the Tea Leaves Extract is as presented in Table 1.

Table 1: Phytochemical analysis of Tea Leaves Extract.

Phytochemicals	Present	Absent
Phenols	++	
Steroids	++	
Carbohydrates		-
Anthocyanins	++	
Flavonoids	++	
Saponins		-

Aqueous Tea Leaves Extract was analysed and the results are presented in Table 1. This analysis showed phytochemicals such as phenols, steroids, flavonoids, anthocyanins are present in the Tea Leaves Extract while, carbohydrates and saponins are absent in the Tea Leaves Extract. This is in line with the branded; 'HighLand Tea' (a product of Kakara Group of Companies, Taraba State-Nigeria) which uses the Tea Leaves for its products, and the other findings [13]. The phytochemicals present in the Tea Leaves Extract could be responsible for the reduction of Cu^{2+} ions and capping of the CuNPs.

3.2. Characterization of Tea Leaves Extract and CuNPs by UV-visible Spectrophotometry

The interaction of NPs with wavelengths of light is dependent on the size, shape and cluster state of NPs. Thus, UV-visible spectroscopy is used for confirming the formation of NPs [13]. In our synthesis, the reduction of Cu ions to CuNPs by Tea Leaves Extract was observed by the change in colour of the reaction mixture (from red to brown then darkened), Figure 2. The mixture (synthesized CuNPs) showed an absorption peak at 400 nm (Figure 3a), confirming the presence of CuNPs, which is in line with other works [12]. The shift in the absorption band from 440 nm of the Tea Leaves Extract (Figure 3b) to 400 nm of CuNPs (Figure 3a) further supports this claim [1, 14].

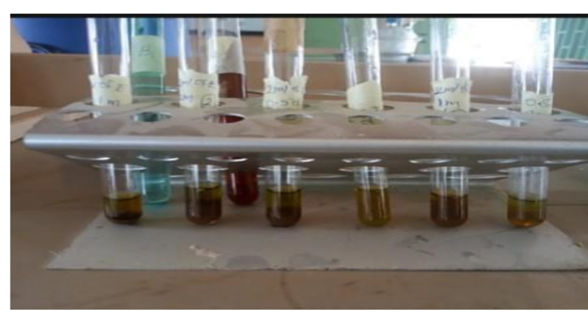
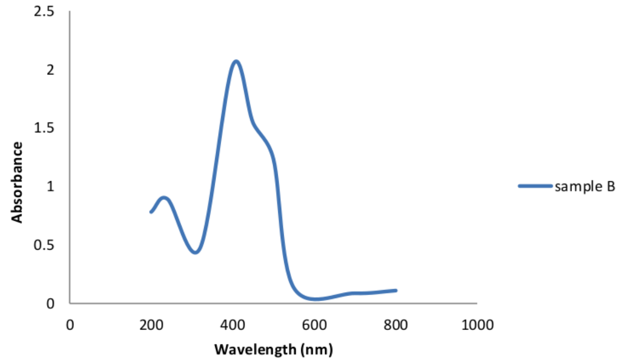
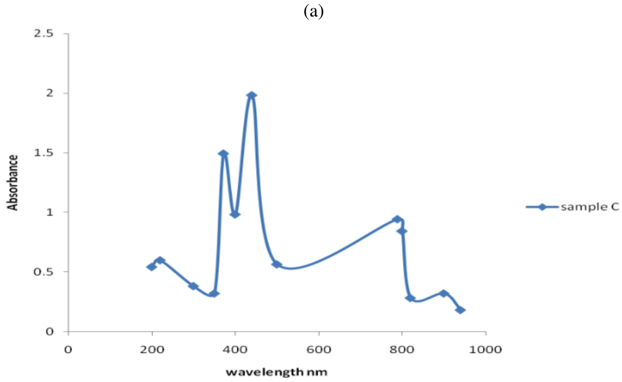


Figure 2: Synthesis of copper nanoparticles.

The functional groups in the Tea Leaves Extract and CuNPs were also analyzed by FTIR spectroscopy. The FTIR spectrum of the Tea Leaves Extract (Figure 4) shows various peaks at; 3200 cm^{-1} (corresponding to O-H), 1540 and 1544 cm^{-1} (corresponding to C=C), while those at 1160 and 1087 cm^{-1} correspond to stretching vibrations of hydroxyl and carboxyl functional groups of phenols and carboxylic acids respectively. The band at 1396 cm^{-1} corresponds to C−O−H bending [15, 16]. The FTIR spectrum of the synthesized CuNPs (Figure 5) on the other hand shows a shift in some of the peaks and the resulting peaks are narrower and less intense, because some of the active compounds in the Extract took part in the reduction of Cu^{2+}



(a)



(b)

Figure 3: (a) UV-visible spectrum of synthesized copper nanoparticles, (b) UV-visible spectrum of Tea Leaves extract.

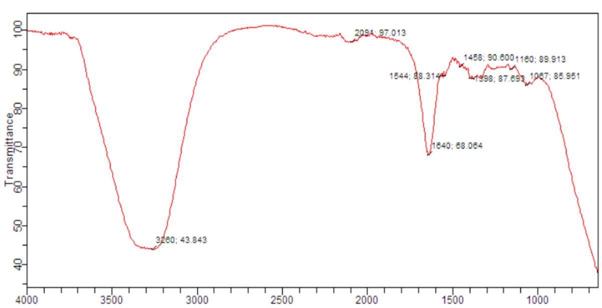


Figure 4: FTIR spectrum of Tea Leaves extract

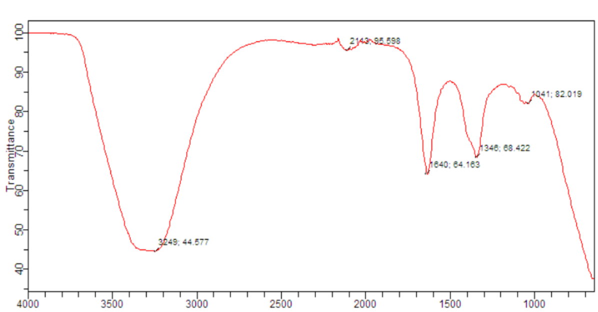


Figure 5: FTIR spectrum of synthesized copper nanoparticles

ions and capping of the CuNPs. The reduction and capping of the CuNPs prepared could be due to the presence of phenols, flavonoids, steroids or anthocyanins groups, which were seen in the IR spectrum (Figure 4). Previous reports on Green synthesis of NPs have shown the presence of these phytochemicals [10].

3.3. Antibacterial Activity of the CuNPs

The antibacterial activity was done in terms of zone of inhibition measured, and was recorded in millimeters using a ruler as contained in Table 2.

Table 2: Antibacterial activity of CuNPs on *Escherichia Coli*.

Microorganism	Nanoparticles (mg/mL)	Sensitivity (34hrs) (mm)	Sensitivity (48hrs) (mm)
Escherichia Coli	0.500	5.00	7.00
	0.250	2.00	4.00
	0.125	0.00	1.50
	0.063	0.00	0.00
	0.031	0.00	0.00

Bacterial strain, *Escherichia coli* was used to study the antimicrobial activity of the CuNPs obtained from Tea Leaves Extract. Table 2 presents the antibacterial activity of the CuNPs on the bacteria in a well diffusion assay. The results showed that CuNPs have very good antibacterial activity against *Escherichia coli*. The inhibition zone diameter is related to the magnitude of activity of CuNPs on microbes. It was observed that, the CuNPs exhibited antibacterial activity at a concentration similar to that of the reference drug (streptomycin). The effects of the CuNPs against bacterial could be due to the dual activity nature of the nano-sized copper and the bioactive agents of the Tea Leaves Extract on the surface of the CuNPs [17, 18].

4. Conclusion

Due to the phytochemicals present in Tea Leaves (*Camellia Sinensis*) Extract; phenols, steroids, flavonoids, anthocyanins etc, which are responsible for the reduction and capping of CuNPs, it use in the synthesis of CuNPs has been developed. Tea Leaves were used in producing copper nanoparticles. The CuNPs synthesized were characterized by UV-visible and FTIR spectrophotometry. The formation of CuNPs was observed by colour change from red to brown (and darkened). The synthesized CuNPs displayed excellent antibacterial activity against *Escherichia coli*. The bioreduction process of the CuNPs is an economic and ecofriendly, simple one-step method, and this green synthetic protocol would be a better alternative to the existing methods. The use of *Camellia Sinensis* in the synthesis of NPs could also expand its market beyond mere Tea production/consumption, providing a means of livelihood for the Mambilla people of Taraba State, of Nigeria.

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