Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 1763-1767
Received: 17-03-2019
Accepted: 21-04-2019

Kanmani R
PG & Research,
Department of Chemistry,
Holy Cross College
(Autonomous), Tiruchirappalli,
Tamil Nadu, India

Kalaiselvi B
PG & Research,
Department of Chemistry,
Holy Cross College
(Autonomous), Tiruchirappalli,
Tamil Nadu, India

Felicita Florence
PG & Research,
Department of Chemistry,
Holy Cross College
(Autonomous), Tiruchirappalli,
Tamil Nadu, India

Amala Infant Joice J
PG & Research,
Department of Chemistry,
Holy Cross College
(Autonomous), Tiruchirappalli,
Tamil Nadu, India

Correspondence
Kanmani R
PG & Research,
Department of Chemistry,
Holy Cross College
(Autonomous), Tiruchirappalli,
Tamil Nadu, India

Synthesis and antimicrobial studies of copper nanoparticles from costus pictus: Green method

Kanmani R, Kalaiselvi B, Felicita Florence and Amala Infant Joice J

Abstract
The present study involves the green and eco-friendly synthesis of CuNPs using the leaves extracts of the plant *Costus pictus*. For CuNPs synthesis the different bio-components present in the leaves extract works as a reducing agents. The synthesized CuNPs were characterized by UV-visible spectroscopy, Scanning Electron Microscopy (SEM), EDAX and FTIR. The CuNPs formed were confirmed by the characteristics surface plasmon resonance (SPR) peak found at 560nm in UV-visible spectra. The morphological study from SEM images, it is confirmed that unsymmetrical spherical size Copper nanoparticles settled on leaves extract residue. FTIR spectrum clearly illustrates the green synthesis of copper nanoparticles mediated by the leaves extract. These CuNPs were investigated for potential use as an antimicrobial agent to inhibit pathogenic bacteria and fungi. Their antibacterial activity was tested on *Escherichia coli* and *Staphylococcus aureus* and the antifungal activity was tested on *Aspergillus niger* and *Candida albicans.* The results showed that CuNPs had a high antimicrobial activity.

Keywords: copper nanoparticles (cunps), *costus pictus* plant extract, characterization, antimicrobial activity, antipyretic activity

1. Introduction
Due to wider applications of Metal nanoparticles, more researchers are attracted to this field. Some applications of nanoparticles are (a) a catalyst in organic chemistry, (b) thermal and optical properties, (c) biological application. In recent years, Synthesis of Metal Nanoparticles using plant leaf extract has attracted attention of many researchers because of availability of materials, inexpensive and process is easy to carry out in any laboratory, use of non-toxic reagent. In the recent years, Copper nanoparticles (CuNPs) have been used in various fields, including agricultural, industrial engineering and technological fields.
Costus pictus is commonly known as spiral ginger, belonging to the family Costaceae. The major attraction of this plant is its stem with spiral leaves and light airy and tissue paper like flowers. Red painted stem enhances the beauty of the glossy leaves and strongly spiralling canes. It is a magical cure of diabetes. Its leaf helps to build up insulin the human body. So, it is commonly known as Insulin plant. Insulin plant was grown in America and is becoming popular in India because of its medicinal value. It is now accepted and used widely as an Ayurvedic medicinal herb. Leaf is traditionally used as antidiabetic, antioxidant, antibacterial, anti-cancer and diuretic. It was reported that fresh leaves contains 18 chemical compounds were identified by using GC-MS. In this study, I report the synthesis of copper nanoparticles from the leaves of *C. pictus*.

2. Materials and Methods
Phytochemical Analysis
Preparation of *Costus Pictus* Leaves Extract
The fresh *Costus pictus* leaves were collected from KK Nagar, in Trichy district. The leaves were thoroughly washed several times using normal water and then followed by distilled water to remove impurities. The cleaned leaves were subsequently dried under sunshade to remove moisture completely, powdered by using mechanical grinder and then stored. The 20 g of powdered plant leaves were taken into a beaker along with 100 ml of methanol and the leaves were soaked methanol at room temperature. The prepared solution was initially filtered through normal filter paper thereby powdered leafy materials will be filtered out. The filtrate was again filtered through Whatman No.1 filter paper to get clear solution. The filtrate was stored at 4°C for future works.

Phytochemical Screening
Phytochemical screening was done by Standard procedure.

Synthesis of Copper Nano Particles
Preparation of plant leaf extract
The 5g of powdered plant leaves were taken into a beaker along with 100 ml of distilled water and transferred to conical flask and allowed to boil at 60 °C for 30 min under reflux condition then it was cooled down to room temperature. The prepared solution was initially filtered through normal filter paper thereby powdered leafy materials will be filtered out. The filtrate was again filtered through Whatman No.1 filter paper to get clear solution. The filtrate was stored at 4 °C for future works.

Synthesis of Copper Nanoparticles
25 ml solution of plant extract was introduced drop wise into 100ml of 1mM (0.001mM) solution of copper sulphate under continuous stirring. After the complete addition of leaf extract, the mixture was kept for incubation for 24hrs. Within a particular time; the green colour solution was changed into straw yellow in aqueous extract and green colour solution was changed into straw green in ethanol extract, which indicates the formation of copper nanoparticles. Then the solution was centrifuged for 15 min at 10,000 rpm and dispersed in double distilled water to remove any unwanted biological materials.

Characterization of Copper Nano Particles
UV-Visible Analysis
The extracts were examined under visible UV-Visible spectrum. The sample is dissolved deionized water. The Nanoparticles were scanned in the wavelength ranging from 190-1100nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 50 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

Fourier Transform Infrared (FT-IR) Spectroscopic Analysis
Spectra were obtained with the aid of an OMNI-samplerattenuated total reflectance (ATR) accessory on a FT-IR spectrophotometer (Perkin Elmer Spectrophotometer system, USA) followed by previous methods with some modification (Liu *et al*., 2006). A small amount of liquid sample was respectively placed directly on sample holder of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000 $cm^{-1}$to 400 $cm^{-1}$and computerized for analyses by using the 21 CFR part 11software. The reference spectra were acquired from the cleaned blank crystal prior to the presentation of each sample replicate. The peak values of FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum.

SEM analysis of copper nanoparticles
Scanning electron microscopic (SEM) analysis was done using VEGA3 LMU machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the copper nanoparticles on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

EDX spectrum
The chemical composition of bio synthesized copper nano particles was revealed by EDX spectrum.

XRD Analysis
X-Ray diffraction results clearly show that Copper nanoparticles formed by both type of leaf extracts are crystalline in nature (Fig 7 & 8). Crystalline size of CuNPs formed was calculated using the Debye-Scherrer equation

$$d = \frac{K\lambda}{\beta \cos\theta}$$

Where D is the crystallite size of CuNPs, λ is the wave-length of the X-Ray source used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 to 1, ϑ is the Bragg angle.

Determination of Antimicrobial Activity
Antimicrobial assay
Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli*, *Staphylococcus aureus* were spread on Nutrient agar plates for bacteria and *Candida albicans* and *Aspergillus flavus* were spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl, 100 µl and 150 µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37 °C for 24 h for the bacteria and at room temperature (30 ± 1) for 24-48 hr. for fungal strains. Each sample was tested in triplicate.

Measurement of zone of inhibition
The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

Results and Discussion
Phytochemical Screening
The results of phytochemical screening were revealed the presence of alkaloids, tannins, carbohydrates, etc. and

flavonoids, phytosterols and phenolic compounds are highly present in *Costus pictus. (Table 4.1)*

Table 4.1: Results of Phytochemical screening of the plant Costus pictus

S. No	Phytoconstituents	Costus pictus
1.	Alkaloids	_
2.	Carbohydrates	_
3.	Glycosides	+
4.	Reducing sugar	_
5.	Saponins	_
6.	Monosaccharides	_
7.	Tannin	_
8.	Proteins & Free amino acids	_
9.	Flavonoids	++
10.	Terpenoids	+
11	Poly saccharides	_
13.	Protein	_
14.	Amino acids	_
15.	Steroids	++
16.	Anthraquinone glycosides	_
17.	Resins	_

N.B: (++) indicates highly positive (+) indicates Positive (-) indicates Negative

Synthesis of Copper Nanoparticle

The formation of Cu NPs was initially confirmed visually. The change in color (Blue to greenish yellow) of the reaction mixture (Fig.1) due to surface Plasmon resonance singularity provides a convenient signature to indicate the formation of CuNPs.

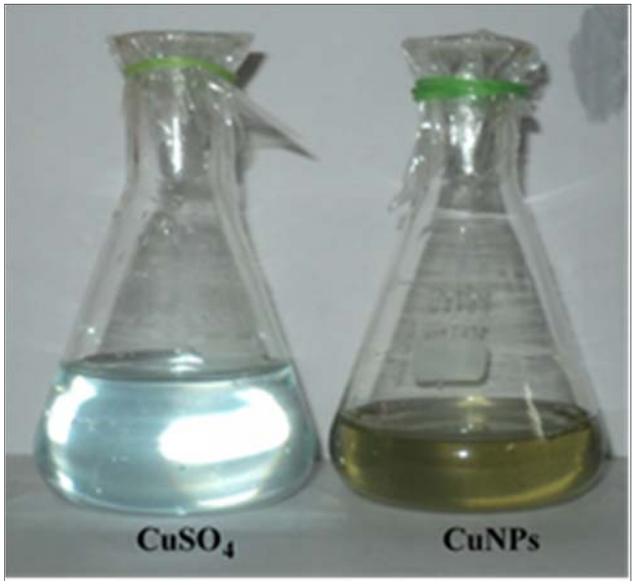

Fig 1. Synthesis of Cu NPs from the leaves of *Costus pictus* by aqueous extract

Characterization of Copper Nanoparticles

UV-Vis absorption spectroscopy

The absorption spectra of aqueous and ethanol extracts obtained from Gymnema sylvestre were compared with the absorption spectra of copper nanoparticles prepared using these extracts in order to reveal the formation of copper phyto-nanoparticles. The absorption spectra of copper phytonanoparticles were recorded after 24 hours after their preparation and exhibited absorbance peaks at 560 nm.

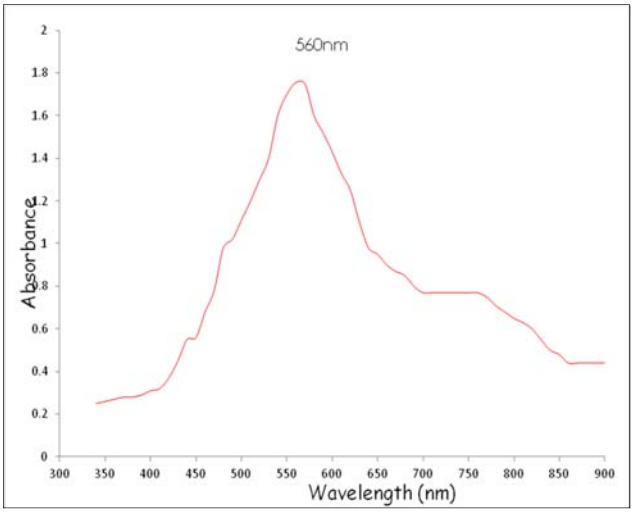

Fig 2: Uv-visble absorption of spectrum of synthesised copper nanoparticles by *Costus pictus*

Fourier Transform Infrared (FT - IR) Spectroscopic Analysis

The result of FT- IR spectrum of synthesized copper nanoparticles using extract of *Costus pictus* was shown in Figure 4.2. Ii is used to analyse the capping, reducing and stabilizing effect of the methanolic leaves extract of *Costus pictus*. The broad peak at 3000cm^{-1} corresponds to the O- H stretching of the Phenols and alcohols. The peak at 1637 cm^{-1} is due to the presence of C=O stretching of aldehydes. The peak value at 1097 cm^{-1} is shows the presence of C-O stretching of alcohols. From the FTIR spectrum of Cu nanoparticles, it can be cofirmed that Cu nanoparticles were surrounded by different functional groups such as terpenoids, alcohols, ketones, aldehydes and carboxylic acid.

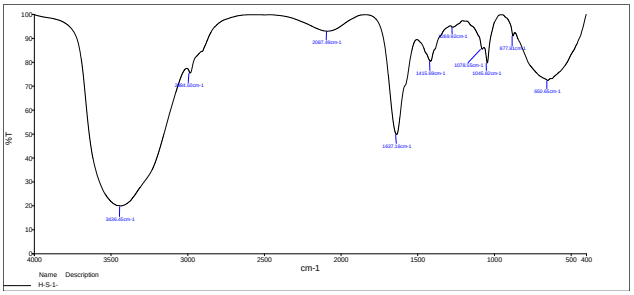

Fig 3: FT-IR spectrum of synthesized copper nanoparticle by *costus pictus*

Scanning Electron Microscopy (SEM)

The surface morphology and size of the nanoparticles were obtained by Scanning Electron Microscopy (SEM) analysis. The Fig shows the CuNPs synthesized by the plant extract of Costus pictus. The electrostatic interactions and hydrogen bond between the bio-organic capping molecules bond are responsible for the synthesis of copper nanoparticles using plant extract. It was shown that spherical and relatively uniform shape of the copper nanoparticles was confirmed in the range of 10-100nm.

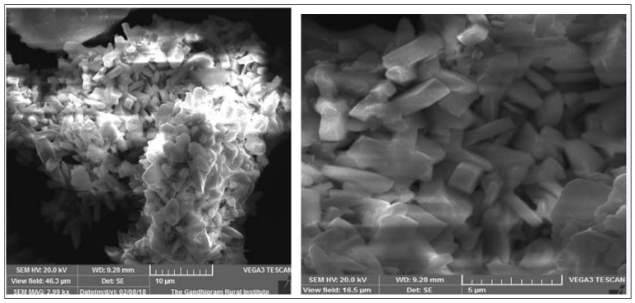

Fig 4: High resolution scanning electron microscopic (SEM)image of copper nanoparticles (CuNPs) (Polydispersed CuNPs ranged between 10-100nms)

EDAX analysis

The elemental composition of the synthesized copper nanoparticles was identified by energy dispersive X-ray (EDAX) analysis. Figure 5. shows the EDAX pattern of synthesized copper nanoparticles by using copper sulphate and the methanolic leaves extract *Costus pictus* which indicates the presence of Copper. Elemental composition is represented in Table 2. Due to the Surface Plasmon Resonance, the copper nanoparticle shows the absorption peaks of higher counts

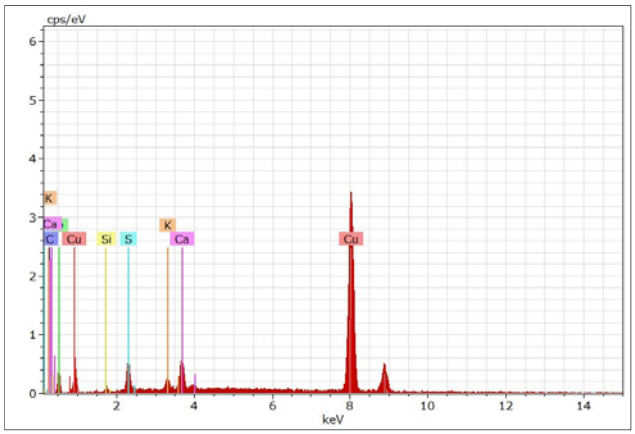

Fig 5: EDAX Spectrum of Copper nanoparticles by *Costus pictus*

XRD Analysis

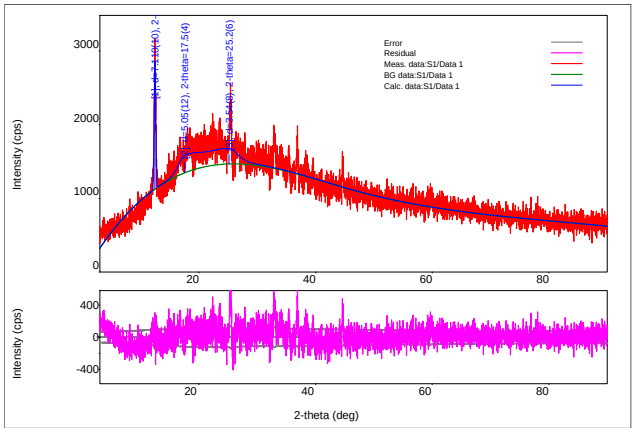

Fig 6: XRD Spectrum of Copper nanoparticles by *Costus pictus*

The particle size and nature of the synthesized copper nanoparticles are determined by using XRD analysis in the angle ranging from 20º to 80º. A thin film of the copper nanoparticle is made by dipping a glass plate in the solution and carried out the X-ray diffraction studies. The diffraction peaks at 12.4º, 17.5º, and 25.2º for synthesized copper nanoparticles using *Costus pictus* as shown in Figure 6.

Antimicrobial activity

The results obtained from the paper disc diffusion assay showed significant reduction in the bacterial growth exhibiting a dose dependent effect (i.e) increase in concentration increased the zone of inhibition. The synthesized copper nanoparticles showed considerable antibacterial activity against gram gram negative bacteria and two fungi. The CuNPs were more active against *Escherichia coli* with IZD of 4.70 mm. The potency of the crude extract was comparable to those of standard antibiotics which are pure substances. Chloramphenicol was used as positive control against the selected bacterial strains. It shows the IZD ranging from 8. 10 mm to 8.50 mm and Fluconazole was used as positive control against the fungal strains and the IZD ranges from 8.30 mm to 8.60 mm.

Microorganisms	$CuSO_4$ (30µl)	Plant extract (30µl)	CuNPs (30µl)	Standard (30µl)
Bacteria				
Escherichia coli (mm)	0.20±0.01	0.70±0.04	4.70±0.32	8.10±0.56
Staphylococcus aureus (mm)	0.30±0.02	0.50±0.03	4.40±0.30	8.50±0.59
Fungi				
Candida albicans (mm)	0.10±0.01	0.20±0.01	2.90±0.20	8.30±0.58
Aspergillus niger (mm)	0.10±0.01	0.30±0.02	2.00±0.14	8.60±0.60

Values were expressed as Mean ± SD

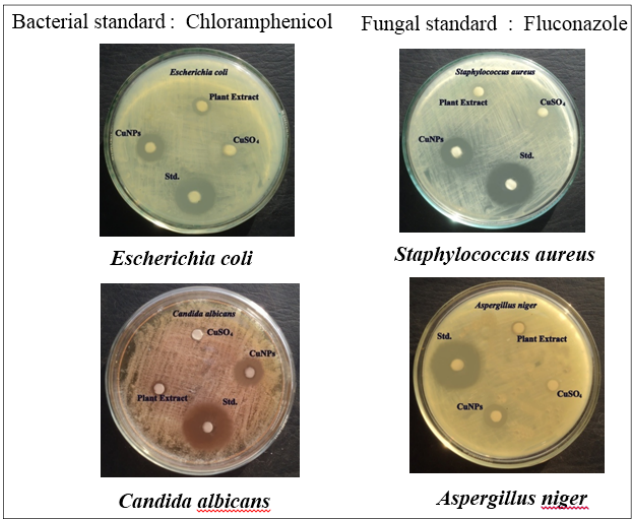

Fig 7: Antimicrobial Activities of Synthesised Copper Nanopaticle of the *Costus pictus*

4. Conclusion

In this study, we have developed eco friendly and environment safe green method for the synthesis copper nanoparticles from *Costus pictus* leaves extract with rapid speed. The leaves extract very much suitable for the synthesis of small sized copper nano particles. The colour changes from light brown to dark brown indicates the presence of different phytochemicals responsible for the reduction, stabilization and capping of copper nanoparticles, which is confirmed by UV-Vis spectroscopy and FT-IR. FT-IR reveals that the phenols and primary amines of proteins are mainly responsible for the reduction and capping of this nanoparticles to prevent agglomeration and provide stability to the medium. The nanoparticles are very small in range between 10-100nms

confirmed by SEM and the total content of copper nanoparticles was identified by EDAX instrument. Further the antimicrobial studies indicated that the nanoparticles are toxic to different types to dry resistant micro organisms. Finally we concluded that the leaves of *Costus pictus* are ideal material for the rapid synthesis of copper nanoparticles and act as a potential antimicrobial agent.

References

1. P Naga Padma1, Syed Thanveer Banu1, Chaitanya Kumari1 S. Studies on Green Synthesis of Copper Nanoparticles Using Punica granatum. 2018; 23(1):1-10.
2. Pawan Kaur, Rajesh Thakur, Ashok Chaudhury. Biogenesis of copper nanoparticles using peel extract of Punica granatum and their antimicrobial activity against opportunistic pathogens. 2016; 9(1):33-38.
3. Valli G, Geetha S. Green Synthesis of Copper Nanoparticles using Cassia Auriculata Leaves Extract. 2016; 02(01):05-10.
4. Muhammad Raffr, Saba Mehrwan, Tariq Mahmood Bhatti, Javed iqbal Akhter, Abdul Hameed, Wasim Yawar M. Masood ul Hasan Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. 2010; 60(1):75-80.
5. Ashtaputrey SD, Ashtaputrey PD, Neha Yelane. Green Synthesis and Characterization of Copper Nanoparticles Derived From Murraya Koenigii Leaves Extract, 2017, 10(3).
6. Rozina R, Shaikh Saeed S, Mirza Manisha R, Sawant and Sushama B. Dare Biosynthesis of Copper Nanoparticles using Vitis vinifera Leaf Extract and Its Antimicrobial Activity. 2016; 8(4):265-272.
7. Gopinath M, Subbaiya R, Masilamani Selvam M, Suresh D. Synthesis of Copper Nanoparticles from Nerium oleander Leaf aqueous extract and its Antibacterial Activity. 2014; 3(9):814-818.
8. Jeyaraman Ramyadevi, Abdul Rahuman. Synthesis and antimicrobial activity of copper nanoparticles. 2012; 71:114-116.
9. Zain NM, Stapley AGF, Shama G. Green synthesis of silver and copper nanoparticles using ascorbic acid and chitosan for antimicrobial applications. Carbohydrate Polymers. 2014; 112:195-202.
10. Jayesh P, Ruparelia Arup Kumar, Chatterjee and Suparna Mukherji. Strai specificity in antimicrobial activity of silver and copper nanoparticles. 2008; 4(3):707-716.