Chemistry Research Journal, 2023, 8(3):45-51
Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Usage of husk of zea mays (corn husk) extract for the green synthesis of copper nanoparticles and its application as antifungal, antimicrobial activities

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Abstract CuNPs involving plant extract is significant work nowadays as the metal plays an important role and also this method is eco-friendly. In the present work, we tried to get extract from husk of a zea mays. Formation of CuNPs was confirmed by UV-Visible spectroscopic absorption and Infra-red absorption spectrometry. The morphology of CuNPs was confirmed by SEM.The formation of CuNPs using husk of zea mays extract was unique work reported so far. Phytochemical test analysis of the husk extract of zea mays was done for the presence of natural products which imparts unique properties to the extract synthesised. Antimicrobial studies and antifungal studies confirmed its potentiality.

Keywords Green synthesis, Copper nanoparticles, husk of a zea mays extract, characterization techniques, Antimicrobial and antifungal activities

1. Introduction

Copper metal is biologically significant and green chemistry deals with the approach of synthesis of CuNPs using less hazardous solvents and chemicals. Biologically –mediated synthesis of nanoparticle by this method was environmentally safe. The production of CuNPs of defined size using including various biological system including bacteria, fungi, plant extracts s. The application in CuNPs in various biological and biomedical applications, such as antifungal, antimicrobial were of more significant nowadays. The husk extract possess the antioxidant properties and contains flavonoids and ferulicacids.

Figure 1: Picture of husk of zea mays

Scope of the work

The green synthesis of metallic nanoparticles has been proposed as a cost-effective and environmentally friendly, alternative to chemical and physical methods. In the green synthesis many types of nanoparticles can be done on applications in industries and medicine. Many plant parts or whole plants have been used for the green synthesis of CuNPs due to the presence of a large number of bioactive compounds. Synthesis of CuNPs has been successful with

extracts of various parts of plant species in plants for green synthesis. The disadvantage of green synthesis time consuming and difficult to control over size, shape and crystal. nanoparticles are not mono dispersed [1,2,3].

2. Materials and Methods

Silver nanoparticles were synthesized according to the chemical reduction method by using husk of zea mays extract. This method can easily be performed in any chemical laboratory and economical, thus a cheaper method when compared with other methods of synthesizing silver nanoparticles [4,5].

3. Experimental Section

Figure 2: Picture of Copper Chloride, husk of zea mays extract and CuNPs

Synthesis of Copper nanoparticles

Copper nanoparticles were synthesized according to the chemical reduction by using husk of zea mays extract. This method can easily be performed in any chemical laboratory and economical, thus a cheaper methods of synthesizing Copper nanoparticles.

Preparation of husk of zea mays extract.

A Fresh Corn is taken and the husk of the Corn is removed. It is well cleaned with ordinary tap water and it is rinsed well distilled water. The Corn husk was cut into small pieces. The pieces of Corn husk is weighed accurately for 5 gm. The weighed corn husk is crushed well and it is transferred to well cleaned 250ml beaker. About 50ml water is measured using 50ml Measuring jar and added to it. This mixture of Corn husk and distilled water is boiled until 5mins. After 5mins the mixture indicates the color changes, then the extract is filtered through the help of whattmann 40filter paper. Then it is collected in a well cleaned beaker used as the reducing agent to reduce the copper in upcoming reactions. In this 5ml of extract is used for the preparation Cu NPs.

Preparation of Copper Chloride solution

About 0.1345 gm of copper chloride is taken from our laboratory and weighed accurately. It is made up into 100 ml standard flask up to the mark using distilled water to obtain 0.01N $CuCl_2$.

Copper nanoparticles synthesis

About 10 ml of freshly prepared copper chloride solution is taken in a well Cleaned 100ml beaker using measuring jar. Then 5 ml of freshly prepared corn husk extract is taken using measuring jar and the extract is poured into the beaker containing copper chloride solution, while adding the extract we observe the colour change in in the solution indicating the formation of CuNps. The solution colour changes into pale green colour. The reaction will be completed within 10mins. This solution is concluded as a sample for following tests.

4. Result and Discussion

UV Spectrum of CuNPs synthesised from husk of zea mays extract.

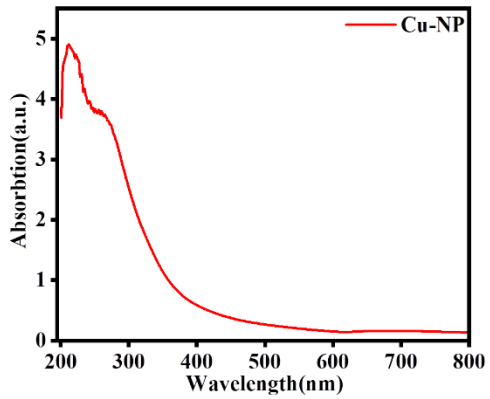


Figure 3: UV Spectrum of CuNPs synthesised from husk of zea mays extract

The reaction was accompanied by colour change due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. In the formation of copper nanoparticles, the test undergone by uv-visible spectroscopy, peak observed at 200 to 300 nm. This confirms the formation of CuNPs.

Phytochemical constituents of husk of zea mays extract

The extract was analysed for the following tests to confirm the presence of the constituents present in the extract [6,7,8].

1. Shinoda's test: 2 ml of extract was dissolved in 5 ml of ethanol and to these 10 drops of dilute hydrochloric acid followed by small piece of magnesium where added. There is no Formation of pink, reddish or brown colour that confirms the absence of flavanoids.

2. Molisch's test:

2 ml of extract was shaken with 10. Ml of water filtered, and filtrate was concentrated. to this 2 drop of freshly prepared 20% of alcoholic solution of alpha naphthol was added.2 ml of conc. sulphuric acid was added ,so as to form a layer below the mixture. No Red-violet ring indicates the absence of Carbohydrates.

3. Tannins:

To 1-2ml of the extract, few drops of 5% w/v FeCL3 solution was added. The light Brown colour indicates the Presence of Tannins

4. Salkowski reaction:

2ml of dry extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by sides of test tube. No red colour indicates the Absence of Steroids.

5. Saponins:

In a test tube containing about 5ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3minutes, No honeycomb like froth indicates the Absence of Saponins. The Tannin present in the extract of zea mays (corn) husk may be responsible for the reduction of copper atom to Nanoparticles. This result obtained from the studies of the phytochemical tests.

Tannin

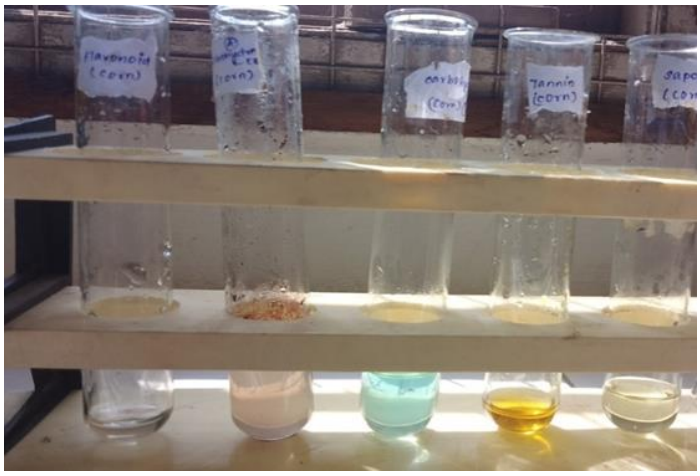


Figure 4: Phytochemical test of the extract from husk of zea mays

Table 1: Phytochemical constituent of *Zea mays* (corn husk) extract

S. No.	Phytochemical constituent present in the extract	Tests	Observance	*Zea mays* (Corn) Husk Extract
1.	Flavanoids	Shinods's	No change	Absence
2.	Carbohydrates	Molisch's	No change	Absence
3.	Tannins	Tannins	Brown colour	Presence
4.	Steroid	Salkowski	No change	Absence
5.	Saponins	Saponins	No change	Absence

IR-Infrared Spectroscopy

In Copper nanoparticles the test undergone by infrared spectroscopy, shows peak at 1550-1600 ,4000, 3500-3700. The correct value of graph at 3500-3700 Cu NPs by the taken sample. The FTIR spectrum of Cu NPs obtained by thermal decomposition process. This analysis was used to determine the functional organic groups in the surface of the nanoparticles generated by oleic acid. Two bands between 3500-3700cm^{-1} can be seen, and they are attributed as symmetric and asymmetric stretching of OH group and terminal groups which correspond to oleic acid. Next Two bands between 1550-1600 cm^{-1} can be seen and they are attributed, as alkane and alkyl group. Then the 1600 -C=C- stretching of alkenes. At 4000 there is a band that indicates the OH groups of amides [9.10].

Figure 5: IR spectrum of copper Nanoparticles obtained from husk of Zea mays

Scanning Electron Microscopy

SEM was used the view morphology and size of the silver nanoparticles.

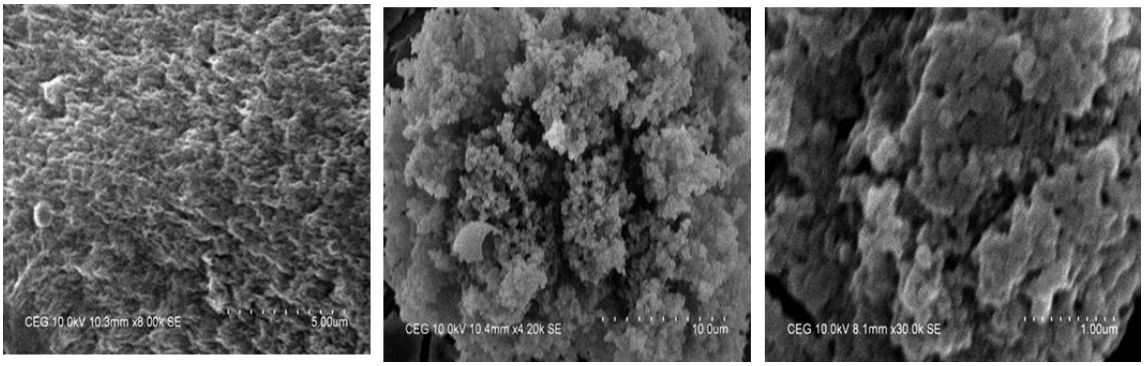


Figure 6: Scanning Electron Microscopy of AgNPs formed from husk of Zea mays extract

SEM analysis is the morphology of the biosynthesized dried NPs. The obtained SEM image shows that the product is mainly made of CuNPs. Cu nanoclusters with size ranges from 150 to 200 nm. However, with high magnification, further observation reveals that these Cu nanoclusters are assembled by smaller NPs, which exhibit the average diameter is about 24.54 nm. No uniform distribution of CuNps. It look shiny in the surface of the Nanocomposites. Agglomeration occurred at the surface of the Nanocomposites. Flakes were seen in the picture.

Antimicrobial Activity

(A) Antibacterial Activity

Determination of Minimum inhibitory concentration (MIC) using Resazurin Microtiter Assay.

Preparation of resazurin solution:

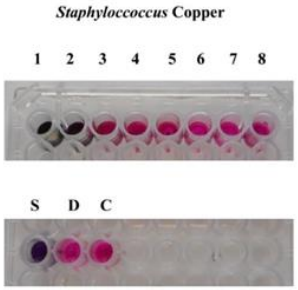
The resazurin solution was prepared by dissolving 270 mg in 40 ml of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

Procedure:

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µl of sample was pipetted into the first well of the plate. To all other wells 50 µl of nutrient broth was added and serially diluted it. To each well 10 µl of resazurin indicator solution was added. 10 µl of bacterial suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 18–24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

Table 2: Antimicrobial studies data

S. No.	Microorganisms/sample	Growth of inhibition										
		1000 µg	500 µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg	STD Steptomycin 10µg	DMSO	Culture
	Copper sample											
1	*Staphylococcus aureus*	-	-	+	+	+	+	+	+	-	+	+



Microorganisms/sample	MIC Value (µg)
Staphylococcus aureus	500

The MIC value for the Cu NPs is Minimum Inhibitor Concentration in the gram-positive bacteria Staphylococcus aureus is 500µg.

Antifungal Activity

Determination of Minimum inhibitory concentration (MIC) using Resazurin Microtiter Assay.

Preparation of resazurin solution:

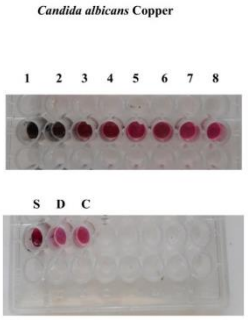
The resazurin solution was prepared by dissolving 270 mg in 40 ml of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

Procedure:

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labelled. A volume of 100 µl of sample was pipetted into the first well of the plate. To all other wells 50 µl of potato dextrose broth was added and serially diluted it. To each well 10 µl of resazurin indicator solution was added. 10µl of fungal suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 18–24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

S. No	Growth of inhibition										
	1000 µl	500 µl	250 µl	125 µl	62.5 µl	31.2 µl	15.6 µl	7.8 µl	STD Ketocanozole 10µg	DMSO	Culture

Copper												
1	*Candida albicans*	-	-	-	+	+	+	+	+	-	+	+



Microorganisms/sample	MIC Value (µl)
Candida albicans	250

From the above data, the synthesized nanoparticle has the ability to act as an antifungal agent.

The MIC value for the Cu Nps is minimum inhibitor concentration in the Antifungal bacteria studies the Candida albicans is 250 µg.

5. Conclusion

We tried the green synthesis of CuNPs using the extract of corn husk. The green synthesis of CuNPs using extract of Corn Husk provides an eco-friendly, cost effective and simple route for synthesis of Copper nanoparticles. The method is found to be efficient in terms of reaction time as well as stability of synthesized CuNPs. The synthesized Cu NPs were characterized using UV, FTIR, SEM and its Phytochemical constituents of the extract and its antimicrobial activities. We want to extend our research in future by using this Cu NPs as a packing materials in food industries.

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