



**BIOSYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL,  
ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF COPPER OXIDE  
NANOPARTICLES (CONPS)**

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**ABSTRACT**

Biosynthesis of nanoparticles has attracted scientist's attention in recent times because of the necessity to develop new, clean, cost effective and efficient synthesis techniques. In particular, metal oxide nanoparticles are receiving greater attention since there is more scope for varied applications. In this study, we describe cost effective and environment friendly technique for biosynthesis of stable copper oxide nanoparticles has been obtained in *Curcuma aeruginosa* by using its powdered rhizome extract. This extract was added to the

3mMol of copper sulphate solution and the change in colour indicates the formation of copper oxides. These biosynthesized copper oxide nanoparticles were characterized with the help of Vis spectrophotometer (UV), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX). The CuO nanoparticles were found to exhibit antibacterial, antifungal and antioxidant activity.

**KEYWORD:** Biosynthesis, nanoparticle, antimicrobial, antifungal, antioxidant, copper sulphate.

**INTRODUCTION**

Nanoparticles have been intensively studied over the last decade due to their characteristics such as physical, chemical, electronic, electrical, mechanical, magnetic, dielectric, optical and

biological properties <sup>[1,2]</sup>. Nanoparticles are measured as building blocks of the next generation of technology with purpose in many industrial sectors. The oxides of transition metals are an essential class of semiconductors and have been intensively studied because of their unique properties for potential applications, such as for solar cells, electronics and photocatalysis. Among various oxides of transition metals, copper oxides have attracted considerable attention due to their interesting photochemical and photomagnetic properties. Copper oxides (CuO) can exist in different stoichiometries and phases such as Cu<sub>2</sub>O and CuO, which have a narrow band gap energy in the range from 1.2 to 2 eV. CuO has been broadly exploited for different applications such as photocatalysts, sensors, lithium ion electrode materials, optical switches and field-emission emitters <sup>[3]</sup>.

In contrast, due to their shape and size-dependent properties, it is obtaining to consider the particular controllability of copper oxide chemical composition, size, shape and surface chemistry to obtain its chemical and physical properties as desired. Currently efforts are towards obtaining the synthesis of copper oxide nanostructures with various morphologies, such as particles, hollow spheres, rod tubes, wires and flowers. Diverse methods have been used to produce copper oxides, such as using templates, microwave-assistance, thermal disintegration and hydrothermal reactions. Development of spotless, biocompatible, non-hazardous and eco-friendly method for nanoparticle synthesis deserves merit. The interest in the field has shifted towards 'green' chemistry and bioprocess approach. These approaches have afocal point on utilization of environment friendly, cost-effective and biocompatible reducing agent for synthesis of copper oxide nanoparticles (CONPs). Review of literature has shown that synthesis of copper oxides using microorganisms and plant extract remains an unexplored area; there are only a very few reports on the use of yeast, fungi, bacteria or plant extract for synthesizing CONPs<sup>[4,5,6]</sup> compared to the great number of publications for other metals.

The present work aims at utilizing *Curcuma aeruginosa* as a bio-factory for the CONPs synthesis. The process is described and different analytical techniques were used including UV-visible spectroscopy(UV-vis), Fourier transform infrared spectroscopy (FTIR), X ray diffraction analysis(XRD), scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX). The CuO nanoparticles were found to exhibit antibacterial, antifungal and antioxidant activity.

## **MATERIALS AND METHOD**

### **Preparation of rhizome extract**

The rhizome was collected from Kottayam in Adivaram. Rhizome was washed several times with distilled water, then cut into small pieces and dried to remove the residual moisture. It was then finely powdered and stored. About 15g of powdered rhizome was transferred into a 150 ml beaker containing 50 ml distilled water and then boiled for 15 min. The extract obtained was filtered and used as a reducing agent and stabilizer. The preliminary phytochemistry of rhizome also was carried out.

### **Synthesis of CONPs**

In a typical reaction procedure, 5ml of rhizome extract was added to 5ml of 0.003M aqueous CuSO<sub>4</sub> solution, with stirring magnetically at room temperature. Within few minutes, blue color became sea green color.

### **Characterization of CONPs**

The bioreduction of copper ion in solution was monitored using UV-visible spectrometer against distilled water as blank. After that, the solution mixture (rhizome extract and copper sulphate) was centrifuged at 5, 500 rpm for 10 min and subsequently redispersed in distilled water and ethanol to get rid of any uncoordinated biological molecule. This process of centrifugation was repeated many times to ensure better separation of the CONPs. The purified dried powders were then used for further characterization FTIR, XRD, SEM and EDAX.

### **Antimicrobial activity of synthesized copper oxide nanoparticles**

The CONPs synthesized using rhizome extract were tested for antimicrobial activity by agar disc diffusion method against pathogenic bacteria, Gram negative and Gram positive. The pure cultures of bacteria were sub cultured on nutrient agar medium. Each strain was swabbed uniformly into the individual plates using sterile cotton swabs. Filter paper disc (Whatman no.3) were sterilized by autoclaving and 20 µl of nanoparticles solution was loaded into each paper disc and allowed to air dry. The dry disc was placed on the previously inoculated agar. It was kept in incubation at 37°C for 24 hours. The different levels of zone of inhibition of bacteria were measured.

### **Antifungal test of copper oxide nanoparticles**

The antifungal activity was tested by well diffusion method. The potato dextrose agar plates were each similarly seeded with each fungal strain. Pure fungal culture was maintained on PDA at 25°C. Fungal strain was swabbed uniformly into each plates and 6mm diameter well created in the plates. Different concentration of nanoparticles solution was added in the well.

### **Copper oxide nanoparticles used in antioxidant activity**

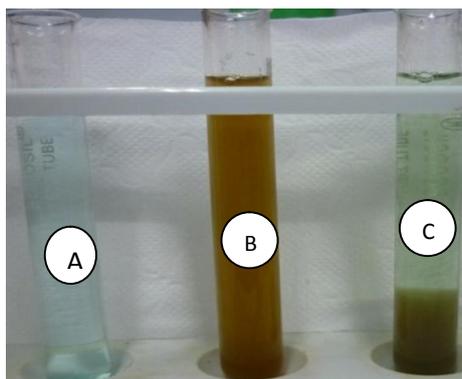
The free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolites of plant and animals. Reactive oxygen species (ROS) react provide protection to living organism from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA strand breaking<sup>[7]</sup>. Several substances from natural sources have been shown to contain antioxidant. The CONPs synthesized using rhizome extract were tested for antioxidant activity. The antioxidant activity was evaluated by DPPH radical scavenging activity. Methanolic solution of 0.5ml DPPH (0.4mM) was added to 1 ml of different concentrations of CONPs solution and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extract served as the positive control. After 30min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows:

$$\text{Scavenging activity (\%)} = \frac{A_{518} \text{ control} - A_{518} \text{ sample}}{A_{518} \text{ control}} \times 100$$

Where  $A_{518}$  control is the absorbance of DPPH radical+ methanol;  $A_{518}$  sample is absorbance of DPPH radical+sample extract/ standard.

### **RESULT AND DISCUSSION**

The formation of CONPS was initially confirmed visually and by using UV –visible spectroscopy technique which has been frequently used to characterize the synthesized metal and metal oxide nanoparticles. Color change of the reaction mixture (fig -1) due to the surface Plasmon resonance phenomenon provides a convenient signature to indicate the formation of CONPs in the reaction mixture<sup>[8]</sup>.



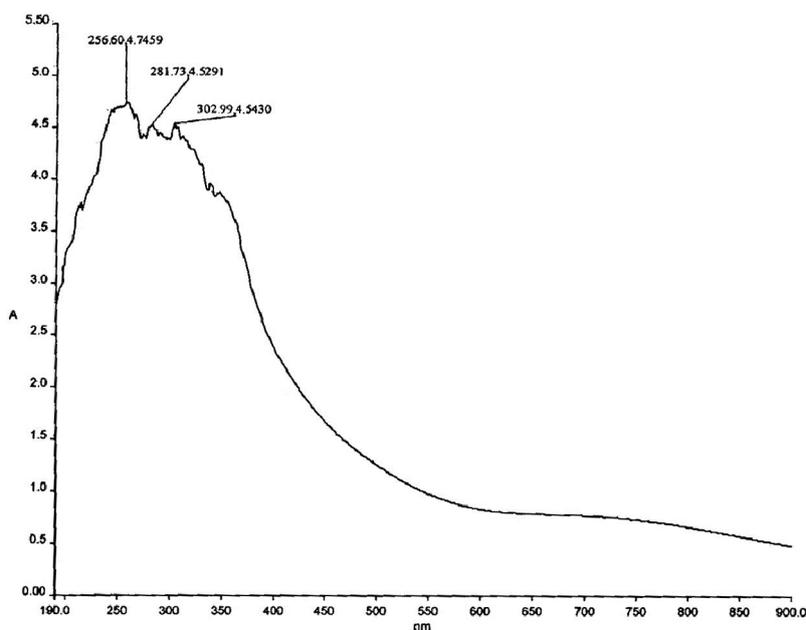
**Fig-1** Tube A- copper sulphate solution, Tube B- contain rhizome extract, Tube C- contain sea green colored copper nanoparticles solution.

**TABLE 1: Change in color of the solution during copper oxide nanoparticles synthesis**

Sr.No	Solution	Color change		Color intensity	time
		Before Reduction	After Reduction		
1.	Rhizome extract	Light brown	Sea green	++	15 minutes
2.	Copper sulphate solution	Light blue			

*Color intensity: - += light color: ++=Dark color: +++=Very dark color*

The UV-absorption spectra recorded (fig-2) shows 260nm which is attributed to the formation of cuprous oxide nanoparticles ( $\text{Cu}_2\text{O}$ )<sup>[9]</sup>. This result clearly suggests that formation of CONPs.



**Fig -2** UV-vis absorption spectrum of CONPs prepared

The XRD pattern of the biosynthesized nanopowder is illustrated in (fig-3). The formation of CONPs in the sample has confirmed the results. This result is in agreement with that UV-visible absorption spectroscopy. Average particle size (d) can be measured by using Scherrer equation:

$$D = k \lambda / (\beta \cos \theta)$$

where D (particle diameter), k is a constant equals 1,  $\beta$  is the full width at half maximum (FWHM) and  $\theta$  is the half diffraction angle.

$$[(9 \times 1.54 \times 10^{-10}) / (\text{FWHM} \times \cos(\theta))] \times [180 / 3.14]$$

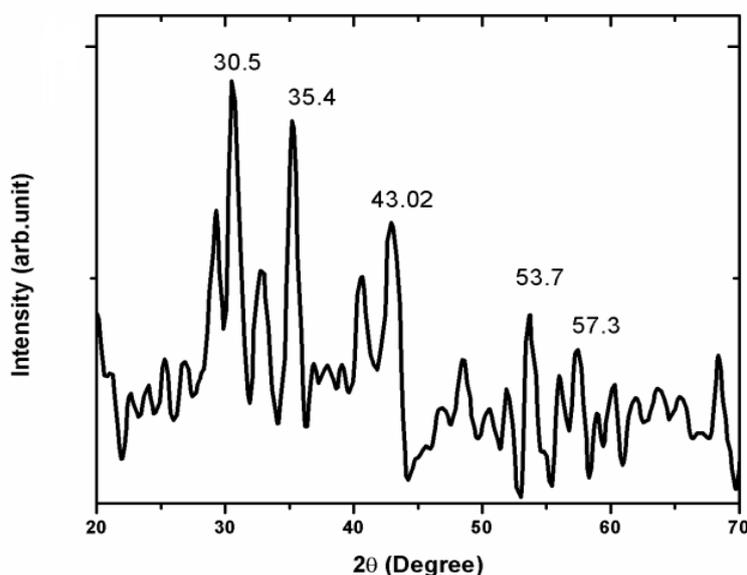
$$2\theta - 30.5 = \text{fwhm} = .304; d = 27.08 \text{ nm}$$

$$2\theta - 35.74 = \text{fwhm} = .405; d = 21.99 \text{ nm}$$

$$2\theta - 26.9 = \text{fwhm} = .186; d = 42 \text{ nm}$$

Take average,  $d = 30.35 \text{ nm}$

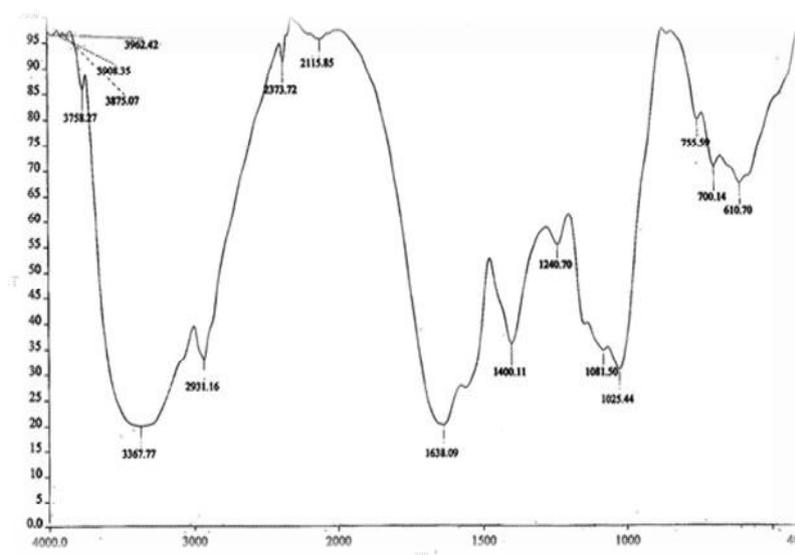
This result is in agreement with that UV-visible absorption spectroscopy and SEM image for the presence and size of copper oxide nanoparticles.



**Fig-3 XRD spectrum of CONPs synthesized from rhizome extract**

FTIR measurements of the synthesized dried CONPs were carried out to identify the possible biomolecules responsible for the reduction, capping of the efficient stabilization of the bioreduced copper nanoparticle. The FTIR spectra of the CONPs are shown in (fig 4). After the synthesis of copper oxide nanoparticles, the absorption peaks at 3368, 1638, 1081 and 1025  $\text{cm}^{-1}$  corresponding to OH, C=C and C-O was observed. The absorption peak at 1638  $\text{cm}^{-1}$

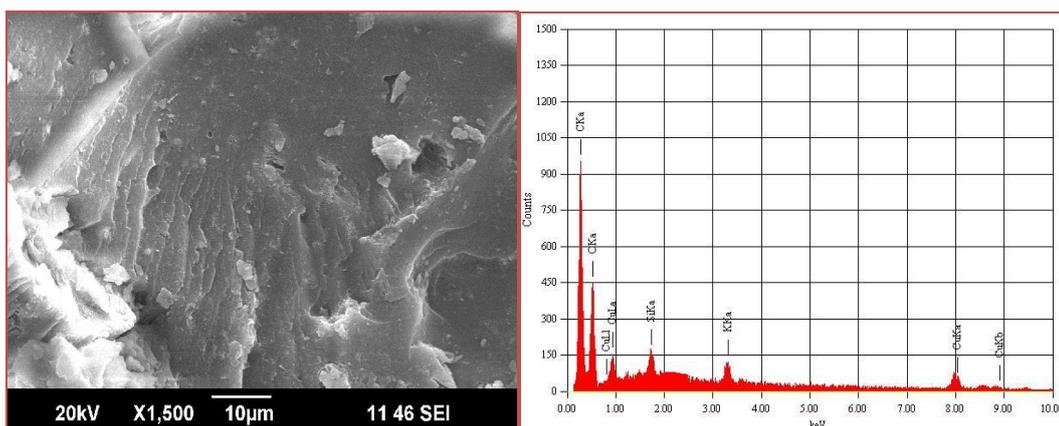
may be assigned to the amide I bond of proteins arising from carbonyl stretching in protein, and the peak at  $3368\text{cm}^{-1}$  is assigned to OH stretching in alcohols and phenolic compounds. The absorption peak at  $1638\text{cm}^{-1}$  is close to that reported for native protein, which suggests that proteins are interacting with biosynthesized CONPs. FTIR study confirmed the presence of possible protein, alcohols and phenols acting as reducing and stabilizing agents.



**Fig.4 FTIR spectrum of CONPs**

The SEM analysis was used to determine the structure of the reaction products that were formed (Fig 5 -A). Scanning electron micrograph of the synthesized CONPs is presented in Fig. 6. Synthesized CONPs do not appear as separate ones but form much larger particles. The observation of such larger CONPs is composed of van der Waals clusters of smaller entities and magnetic interface among the particles.

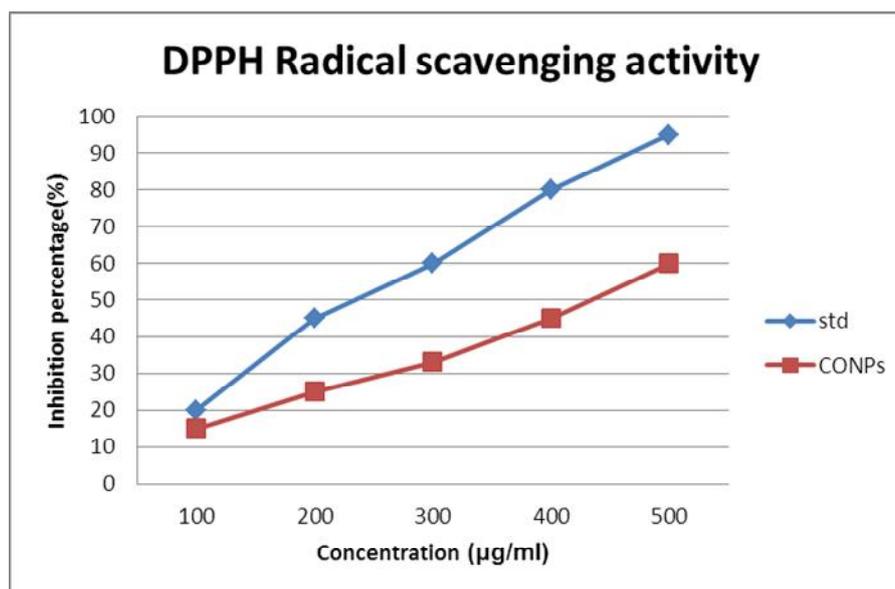
The EDAX spectrum explains the surface atomic distribution and chemical composition of nanoparticles (Fig 5 -B). Quantitative measuring results obtained from EDAX analysis reflect the purity of copper. The other signals of K, C, Si, O are also recorded. The K and Si signals are expected from rhizome extract of curcuma. Potassium (K) plays an important role in many physiological processes [10,11]. Silicon (Si) is the second most abundant element in soil after oxygen. Plants absorb most of silicon in mono silicic acid form. Despite Si being ubiquitous and prominent constituent of plants, it is still widely not recognized as essential nutrients for plants [12]. It is also proved to be beneficial for better plant growth and development [13]. Silicon can improve plant growth and tolerance to biotic and abiotic stresses [14,15].



**Fig.5 A. SEM image of as prepared CuO nanoparticle B. EDX of biosynthesized CONPs**

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by decrease in its absorbance at 517 nm, which is induced by antioxidants.

The percentage of DPPH radical scavenging activity of ethanolic solution CONPs is presented in (fig-6). The free radicals in DPPH can be neutralized by the antioxidants present in CONPs by transferring either their electrons or by hydrogen atoms to DPPH thereby changing the color from purple to yellow-colored diphenylpicrylhydrazine. The  $IC_{50}$  value of biosynthesized CONPs and Ascorbic acid were found to be 510µg/ml and 220µg/ml respectively.

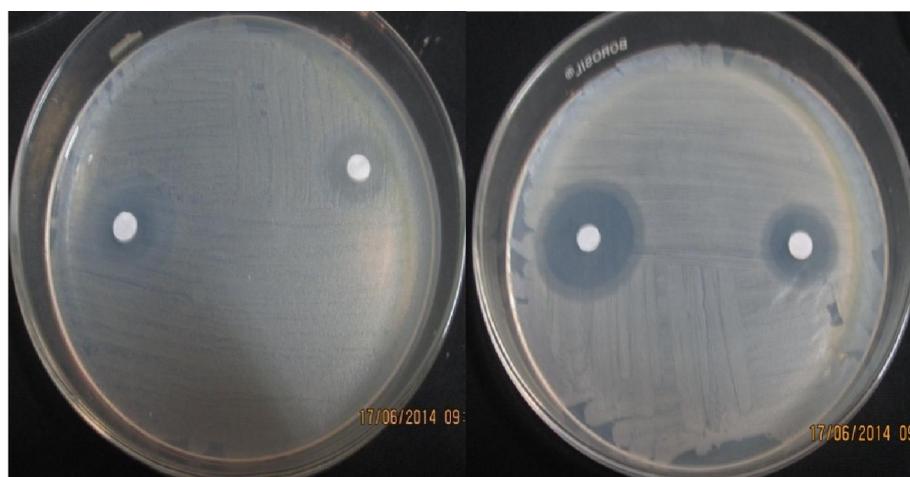


**Fig-6. Percentage of DPPH radical scavenging**

Biosynthesized CONPs were analyzed for their antimicrobial activity against both Gram positive and Gram negative bacteria by disc diffusion method (fig-7). The greatest inhibition zone was observed against *Staphylococcus aureus* in 19mm. Bio synthesized CONPs had significant antimicrobial activity on both Gram positive and Gram negative classes of bacteria, which may be attributed to the greater abundance of amines and carboxyl group on their cell surface and greater affinity of copper ions toward these groups<sup>[16]</sup>. Biosynthesized CONPs exhibit efficient antibacterial property due to their extremely large surface area, which provides better contact with microorganisms. Copper ions released subsequently may bind with DNA molecules and lead to disorder of the helical structure by cross linking within between the nucleic acid strands. Copper ions inside bacterial cell also disrupt biochemical processes<sup>[17]</sup>.  $\text{Cu}^{2+}$  and  $\text{Ag}^+$  ions were also studied since using disrupt the bacterial cell membranes and gain entry in order to disrupt enzyme function. Indirect effects through changes in the surrounding charge environment also have an impact on the effectiveness of nanoparticulate metals against microorganisms<sup>[18]</sup>. It is also possible those copper ions released from the nanoparticles may attach to the negatively charged bacterial wall and rupture it, there by leading to protein denaturation and cause cell death<sup>[19,20,21]</sup>.

**Table -2: Antibacterial activity of copper oxide nanoparticle against gram positive and gram negative bacterial pathogen**

Bacterial pathogen	Zone of inhibition(mm)	
	CONPs	Antibiotic
E.coli(G-ve)	13mm	15mm
Staphylococcus aureus	19mm	26mm
Klebsiella pneumoniae	12mm	14mm
Bacillus cereus	10mm	15mm



**Fig 7-Antibacterial assay:zone of inhibition against *E.coli* and *Staphylococcus aureus***

The antifungal activity of CONPs was examined against *Aspergillus niger* and *A.fumigatus* by using well method. The radial diameter of inhibition zone of *A.niger* and *Afumigatus* by CONPs are 17mm and 18mm respectively.

**Table-3: Antifungal activity of copper oxide nanoparticle against**

Sample	<i>A. niger</i> (mm)	<i>A. fumigatus</i> (mm)
Cu- O	13	13
	16	18
	17	18
	15	17
	14	16
	13	18



**Fig 8-Antifungal assay: zone of inhibition against *Aspergillus niger* and *Aspergillus fumigatus***

## CONCLUSION

The present work is the first report of an eco-friendly and convenient method for the synthesis of CONPs using rhizome (*Curcuma aeruginosa*) extract. No chemical reagent or surfactant template was required in this method, which consequently enabled the bioprocess with the advantage of being environment friendly. The created nanoparticles were characterized by UV-vis, TEMEDX, XRD, and FTIR measurement and showed good antimicrobial and antifungal activity. This can be added to MS medium against the fungal contamination. This technique can be a promising method for the preparation of other metals and metal oxide nanoparticles and can be very valuable in environmental, biotechnological, pharmaceutical and medical application.

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