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Green Synthesis, Characterization and Antimicrobial Properties of Copper Nanoparticles Using Plant Leaf Extracts

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Abstract

In this study, plant extracts from Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica, and Citrus sinensis are used to create copper nanoparticles (Cu NPs) from a copper nitrate solution in an environmentally friendly manner. Atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray diffraction (XRD), and UV–vis spectrophotometry were used to characterize the Cu NPs. Using UV–vis spectrophotometry analysis, the growth and stability of the reduced copper nanoparticles in the colloidal solution were monitored. Scherrer's equation was used to analyze the line width of the refractive peak in the XRD pattern in order to calculate the average diameter of the copper nanoparticles. According to AFM studies, O. tenuiflorum, S. cumini, C. sinensis, S. tricobatum, and C. asiatica formed copper nanoparticles with average diameters of 28 nm, 26.5 nm, 65 nm, 22.3 nm, and 28.4 nm, respectively. Copper nanoparticles were detected by SEM analysis of the stable brown samples; the samples treated with silver nitrate showed the most evenly distributed particles. The well diffusion method was used to assess the silver bio-nanoparticles' antibacterial capabilities against Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus. Interestingly, the copper nanoparticles made from extracts of S. tricobatum and O. tenuiflorum had the strongest antibacterial activity against 30 mm of S. aureus and 30 mm of E. coli, respectively. The Cu NPs generated using this process have potent antibacterial activity against harmful bacteria. Copper nanoparticles are therefore essential to nanotechnology and nanomedicine.

Keywords: *Green Synthesis, Silver nanoparticles, UV-vis spectrophotometer, XRD, AFM, SEM, Antibacterial Activity.*

Introduction:

The Substances with at least one dimension between 1 and 100 nanometers are referred to as nanomaterials (NMs). As a branch of nanotechnology, bionanotechnology is the focused fusion of biological and nanoscale techniques for the biological production of NMs. Small materials with sizes ranging from 1 to 100 nm, known as nanoparticles (NPs), have unique properties that set them apart from their bulk counterparts [1]. One important characteristic that makes it easier for them to be used in a variety of industries, such as the food, electronics, chemical, and medical sectors, is their high surface-area-to-volume ratio. There are several physical, chemical, and biological techniques for creating NPs (Figure 1).

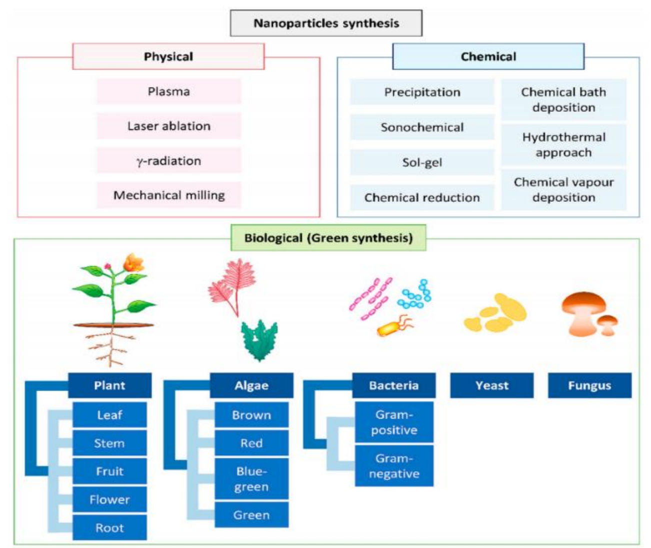


Figure 1. Conventional methods used for nanoparticles synthesis

The Utilizing microbial enzymes or plant phytochemicals, biological synthesis combines physical and chemical techniques with biological principles (such reduction and oxidation) to produce nanoparticles. Solid objects having all dimensions within the nanoscale [2] are called nanoparticles (NPs), and they have the power to drastically change a material's physicochemical characteristics in comparison to its bulk counterpart. Their chemical composition, biological interactions, size, and form can all affect their functions. NMs are widely used in many different industries because to their large surface area to volume ratio [3]. Because of their positive effects on a number of economic sectors, including consumer products, energy, transportation, cosmetics, pharmaceuticals, antimicrobial agents, and agriculture, NMs have recently attracted a lot of interest. Currently, a number of physical and chemical methods have been effectively used to demonstrate the creation of inorganic NPs. Nevertheless, conventional chemical approaches are often inefficient, harmful to the environment, expensive, and prone to toxicity. Therefore, extracellular or intracellular biological synthesis from higher plants or bacteria has become the favored method. The importance of biological synthesis is becoming more widely acknowledged.

For the most part, nanoscience has only been applied theoretically in agriculture. It has the potential to significantly impact illness detection, nutrient absorption, and delivery from the outset. By facilitating the tailored administration of pesticides to fight diseases, it can also increase productivity and improve our understanding of the "biology of diverse crops" [4]. Numerous exploratory research have been conducted in the last ten years to evaluate the observable impacts of nanotechnology on agricultural improvement. Improved seedling growth, germination rates, photosynthetic performance, nitrogen metabolism, protein content, mRNA expression, and changes in gene expression have all been demonstrated by recent studies of NPs in a variety of crops, including corn, wheat, ryegrass, alfalfa, soybean, tomato, radish, lettuce, spinach, onion, pumpkin, bitter melon, and cucumber [5]. Determining the requirement of particular trace elements in plant economies has been the focus of significant work in recent years. The creation of nanoparticles frequently uses metals like copper, iron, zinc, gold, aluminum, palladium, silver, titanium, and fullerenes. Ag NPs, Au NPs, ZnO, and CuO NPs have been the main focus of biosynthetic research up to this point. Because copper-based nanoparticles are affordable, readily available, and have properties similar to those of other metallic NPs, they are very desirable. They are used as catalysts, antibacterial agents for hospital equipment coatings, sensors, and extremely durable materials in heat transfer systems [6].

Since technology is still in its early stages of development and is expected to be widely used worldwide, it is becoming more and more important to include green chemistry concepts into the creation and application of new materials. Innovative "design principles" for creating high-efficiency nanoscale materials that are safe and environmentally sustainable may result from the strong correlation between chemical structure and functional groups that is unique to nanomaterials and from improved understanding of crucial data for lifecycle evaluation of such methods [7]. These plants' molecules, cells, and organs have been bioengineered to produce innovative nanomaterials with significant long-term advantages. Green nanotechnology offers a chance to lessen negative consequences. As seen in Figure 2, it addresses current environmental problems by lowering or eliminating pollution, which has a proactive impact on the design of nanomaterials or products.

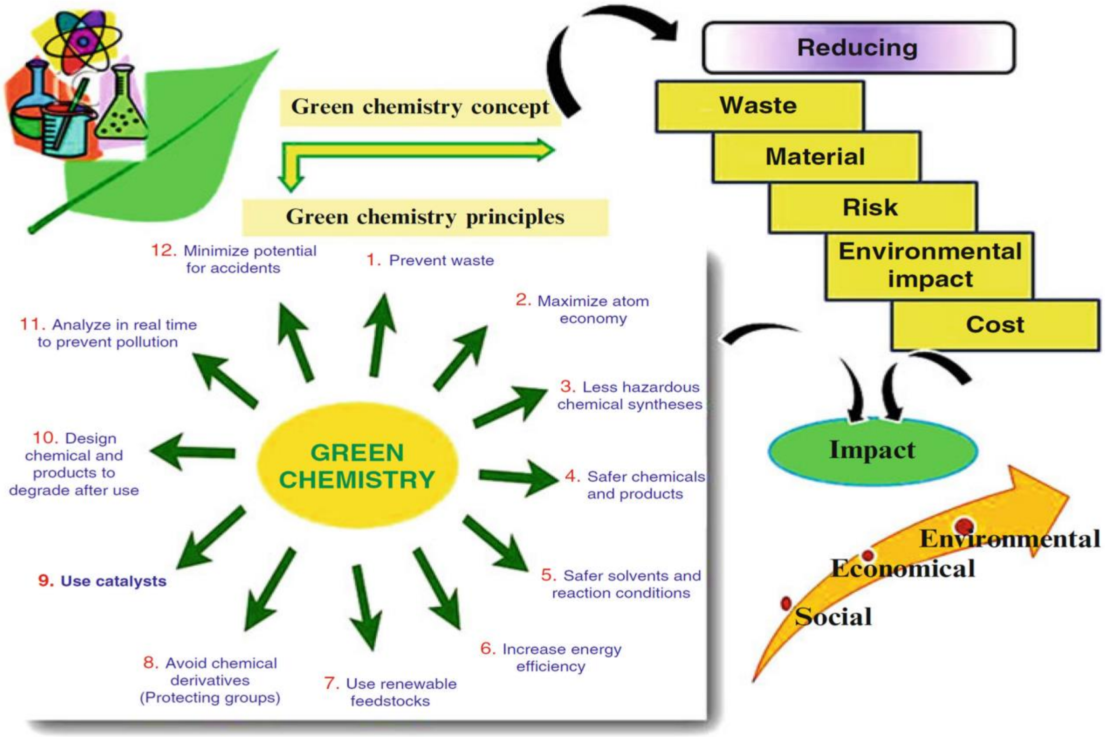


Figure 2: Schematic exemplification of green chemistry combination in metal nanomaterials cloning

Copper Nanoparticles

Recent developments in metallic and metal oxide nanoparticles (NPs) have greatly enhanced biosensing, imaging, diagnosis, and treatment in the biomedical field. Of them, copper (Cu) is the most cost-effective metal, offering superior cost-efficiency when compared to silver (Ag) and gold (Au). Moreover, Cu NPs are efficient catalysts that produce high outputs with simple product separation, enabling repurposing [8]. In reality, the human body needs trace amounts of Cu in addition to vital enzymes. For example, this trace element is incorporated into enzymes like tyrosinase, cytochrome oxidase, and superoxide dismutase. However, at the cellular, organ, and systemic levels, Cu free ions may be harmful to human health. Therefore, controlling Cu ions in living things is essential [9].

Preparation of Biological extracts for Copper Nano particle synthesis

Preparation of Microbial Extracts: According to research, a common procedure is to cultivate microorganisms in a suitable broth medium and then incubate them on a rotary shaker for a predetermined number of days at a temperature and rpm that are particular to the microbe. After that, the cultures are centrifuged for the proper amount of time and at the proper rpm. Cu NPs are synthesized using the resultant supernatants. Bacteria are cultured at 37 °C for 24 hours in an incubator shaker running at 1550-200 rpm, whereas fungi are incubated on a rotary shaker at 200 rpm for 10 days at 28°C [10].

Preparation of Botanical Extracts: Researchers claim that plants of interest are collected from easily accessible areas and thoroughly cleaned using distilled water and tap water to remove any dirt. After two weeks of shade drying, they are processed into a powder in a home blender. A known mass of the dry powder is boiled for two to three minutes at 70 to 80°C with a predetermined volume of distilled water to create the plant broth. After filtering, the resultant infusion is used as a stabilizing and reducing agent [11].

Fabrication of Nanoparticles Using Microorganisms: According to survey research, prokaryotic organisms—specifically, bacteria—are used as agents for the synthesis of nanoparticles because of their simplicity of culture, quick generation time, mild experimental settings (temperature, pressure, and pH), extracellular production, and simple downstream processing. As a result, it has become important in the production of nanoparticles. The silver-resistant bacteria Morganella morganii RP42 and Morganella psychrotolerans, which are responsible for the formation of Cu NPs in the 15-20 nm range, reported that they had isolated CuO NPs from the midgut of Stibara sp., an insect, using a gram-negative bacterium from the genus Serratia. Using non-pathogenic Pseudomonas stutzeri, a fast biological synthesis method was used to create spherical Cu NPs with a size range of 8–15 nm. According to Usha et al. [63], Streptomyces sp.-synthesised CuO NPs may be utilized to create antimicrobial textiles for usage in hospitals with the goal of preventing or minimizing illnesses caused by harmful bacteria. Using Escherichia coli, Singh et al. [64] reported the biological production of CuO NPs with different sizes and morphologies. Cubic Cu NPs with a size range of 50-150 nm were produced by electroplating wastewater using soil-dwelling Pseudomonas stutzeri [12].

Mechanism of Copper Nanoparticle Formation: Much study has been done on the specialized functions of phytochemicals as well as the bioreduction of metal nanoparticles using a mixture of biomolecules found in plant extracts (such as enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids like citrates). Using infrared spectroscopy, the phytochemicals involved have been determined to be terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Quinones, organic acids, and flavones are the main water-soluble phytochemicals that aid in instant reduction. Tree-derived natural hydrocolloids, or gums, are a new class of biomaterials used in the synthesis of nanomaterials. They serve as capping and reducing agents during the creation of nanoparticles. Different bacteria have distinct ways of forming nanoparticles. On the surface or inside microbial cells, they take up target ions from their surroundings. With the aid of enzymes generated by cellular activity, they subsequently reduce the metal ions to nanoparticles. The reduction of metal ions is facilitated by the electrostatic contacts between the ions and the negatively charged cell wall from carboxylate groups in the enzymes. The metal ions then continue to develop through accumulation and reduction [13].

Characterization of Copper Nanoparticle:

The X-ray diffraction, Fourier transform infrared spectrum analysis, UV–visible absorption spectroscopy, and microscopy techniques like transmission electron microscopy [TEM], scanning electron microscopy [SEM], and atomic force microscopy are used to examine the generated nanomaterial. Evaluation of UV-Visible Spectra: According to the results, different salts can produce different nanoparticles, which show clear peaks at 24-hour intervals with varying absorptions using UV-visible spectroscopy. There are noticeable absorption peaks in the 200–800 nm range for Cu Nps. An obvious sign of nanoparticle production is a discernible gradual increase in the characteristic peak associated with the lengthening of reaction time and the concentration of biological extracts and salt ions. Peaks characteristic of the surface plasmon resonance linked to nanoscale particles can be seen in the UV-vis absorption spectrum [14].

The X-ray diffraction (XRD) Analysis:

Technique used to establish the metallic nature of particles gives information on translational symmetry size and shape of the unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms are located from peak intensities [15].

XRD patterns were calculated using X‘per Rota flex diffraction meter using Cu K radiation and $\lambda = 1.5406$ A°. Crystallite size is calculated using Scherrer equation

$$CS = K\lambda / \beta \cos \theta$$

Where CS is the crystallite size
Constant [K] = 0.94
β is the full width at half maximum [FWHM]
Full width at half maximum in radius [β] = FWHM x π/180
λ = 1.5406 x 10-10, Cos θ = Bragg angle.

Fourier Transform Infrared [FTIR] Spectroscopy: Determines the properties of corresponding functional groups and structural features of biological extracts interacting with nanoparticles by comparing the infrared intensity to the wavelength [wave number] of light. The resulting spectra clearly indicate the known relationship between the optical characteristics of nanoparticles, specifically the resonance wavelength, extinction cross-section, and scattering-to-absorption ratio, depending on the size of the nanoparticles [16].

Electron Microscopic techniques: The mostly utilized for nanoparticle morphology analysis. Within the given range, their images reveal the size distribution and morphology of colloidal particles. The produced nanoparticles showed a rather homogeneous size and shape, according to SEM pictures. By focusing on the sample's surface, scanning electron microscopy (SEM) can reveal details about the morphology and topography of the nanoparticles. Three-dimensional (3D) images are produced using it. These methods also make it easier to measure the average particle size. According to Vishveshvar et al., CuO NPs made from the leaves of the Ixiro coccinea plant have a spherical shape and an average size of 300 nm. They also show a tendency to clump together. Similarly, instead of the usual spherical shape, CuO NPs made from mint leaf extract had uneven geometries with size fluctuations similar to nanosheets [17].

The compared to SEM, transmission electron microscopy (TEM) provides greater magnification and resolution. Despite producing flat (2D) images, it offers insights into the interior structure, producing more accurate data regarding the size, shape, and crystallography of nanoparticles. Following the green synthesis of copper nanoparticles using extract from Gloriosa superba, Naika et al. found spherical CuO NPs in the TEM images that ranged in size from 5 to 10 nm. Similarly, the biosynthesis of copper nanoparticles mediated by Portulaca oleracea was carried out, and the extract's efficacy as a stabilizing agent was assessed by observing the TEM images at two extract doses. The results show that NP size decreases from 14 nm to roughly 7–10 nm when extract concentration is increased from 1 mL to 3 mL, producing spherical-shaped CuNPs. For characterisation, energy dispersive X-ray spectroscopy (EDX) is another analytical technique used. For analytical purposes, this method takes advantage of the fact that every element has a different atomic structure, which results in a distinct spectrum of peaks on the X-ray spectrum. After Vijay Kumar et al. carried out the biosynthesis of CuO NPs using aloe vera leaf extract, EDX examination revealed the NPs' chemical makeup, showing an atomic proportion of 54% for copper and 45% for oxygen [18,19].

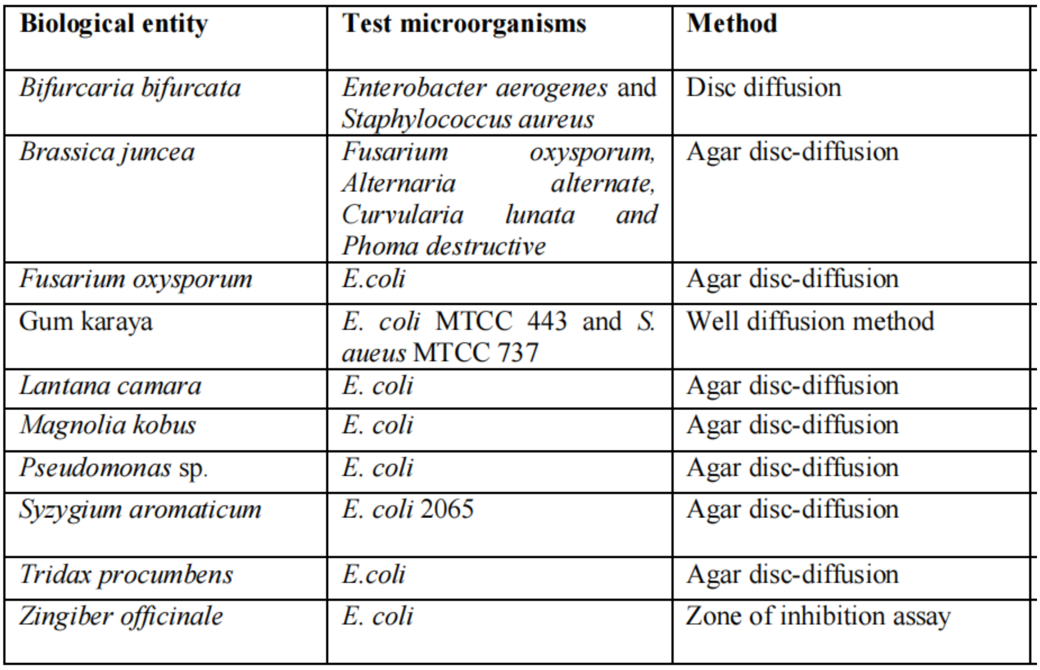
Antimicrobial Activity:

The Copper has been used for many years as an antimicrobial agent because it has a strong antibacterial effect and may effectively reduce the concentration of germs by 99.9%. Copper has been approved for registration by the US Environmental Protection Agency (EPA) as a powerful antibacterial that can reduce some dangerous bacteria linked to potentially fatal microbial diseases. Gram-positive and gram-negative bacterial strains are both susceptible to the broad antibacterial activity of copper nanoparticles (Cu Nps). When it comes to pathogenic species including *E. coli*, *Bacillus subtilis*, *Vibrio cholera*, *Pseudomonas aeruginosa*, *Syphilis typhus*, and *Staphylococcus aureus*, copper oxide (CuO) nanoparticles are a promising antibacterial agent [20]. After 24 hours of growth, the Cu Nps made from plant extracts of Magnolia, Syzygium aromaticum, and Tridax procumbens showed increased antibacterial activity against *Escherichia coli*. The Cu Nps made with Zingiber officinale extract showed a 15 mm inhibition zone against *E. coli*. When tested against *S. aureus* MTCC 737 and *E. coli* MTCC 443, very stable CuO Nps made from gum karya shown significant antibacterial activity.

In contrast to the larger manufactured CuO nanoparticles (7.8 ± 2.3 nm), the smaller dimension of CuO nanoparticles (4.8 ± 1.6 nm) produced a maximal inhibitory zone. With inhibition zones of 14 mm and 16 mm, respectively, CuO Nps made with brown algae extract and measuring 5-45 nm showed strong antibacterial activity against *Staphylococcus aureus* and *Enterobacter aerogenes*. When compared to bigger CuO nanoparticles (7.8 ± 2.3 nm), the 4.8 ± 1.6 nm CuO Nps produced from the microbes *Fusarium oxysporum* and *Pseudomonas* demonstrated good stability and strong antibacterial activity on both types of gram bacteria. Following a 24-hour culture in shake flasks, it was discovered that the nanoparticles made using Magnolia kobus leaf extract had a stronger antibacterial effect on *E. coli* cells [22-25].

The additionally, TEM pictures verified that copper colloidal solutions made by a polyol approach with copper oxalate as a precursor produced nanoparticles with an average size of 6 nm. This result showed that the fungus Corticium salmonicolor was an efficient anti-pink disease treatment for rubber trees. Evidence demonstrated that when a single representative strain of E. coli was used to evaluate the antibacterial capabilities of copper and silver nanoparticles, Cu Nps demonstrated better antibacterial qualities than silver. Cu Nps, which had a particle size of 3-10 nm and were produced by chemically reducing Cu^{2+} with cetyl trimethyl ammonium bromide and isopropyl alcohol, demonstrated strong antifungal activity against plant-pathogenic fungi like Fusarium oxysporum, Alternaria alternata, Curvularia lunata, and Phoma destructive. Additionally, Cu Nps produced by the polyol method with copper acetate hydrate reduced in the presence of tween 80 demonstrated antimicrobial activity against fungal pathogens such as *Aspergillus flavus*, *Aspergillus niger*, *and Candida albicans*, *as well as Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [27, 28]. Table 1 classifies the antibacterial properties of Cu nanoparticles on microorganisms.

Table 1. Antimicrobial effect of biologically synthesized Cu Nps on various microbes



Biological entity	Test microorganisms	Method
Bifurcaria bifurcata	*Enterobacter aerogenes* and *Staphylococcus aureus*	Disc diffusion
Brassica juncea	*Fusarium oxysporum, Alternaria alternate, Curvularia lunata and Phoma destructive*	Agar disc-diffusion
Fusarium oxysporum	*E.coli*	Agar disc-diffusion
Gum karaya	*E. coli* MTCC 443 and *S. aueus* MTCC 737	Well diffusion method
Lantana camara	*E. coli*	Agar disc-diffusion
Magnolia kobus	*E. coli*	Agar disc-diffusion
Pseudomonas sp.	*E. coli*	Agar disc-diffusion
Syzygium aromaticum	*E. coli* 2065	Agar disc-diffusion
Tridax procumbens	*E.coli*	Agar disc-diffusion
Zingiber officinale	*E. coli*	Zone of inhibition assay

Conclusion:

The manufacture and applications of copper nanoparticles are the main topics of this research. Other metallic nanoparticles, such as gold and silver, which are in great demand because of their affordability and accessibility, have been replaced by copper. Green synthesis has gained attention because it is thought to be more reliable, eco-friendly, and straightforward than conventional techniques. For green synthesis, a variety of plant and peel extracts have been successfully used. The presence of bioactive chemicals is essential for stabilizing and reducing copper nanoparticles. The resultant NPs are then described by means of a number of analytical methods, such as TEM, SEM, UV-visible spectroscopy, and others. All of these artificially produced NPs are thought to have important uses in a variety of fields, opening up a bright future for nanotechnology. Copper nanoparticles' biological creation and analysis have shown both positive and negative effects on plants and microbes. Research on the toxicity of nanomaterials is still being conducted, with the goal of revealing the wide-ranging applications of copper nanoparticles in agriculture and successfully resolving its problems.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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