BHARATA MATA COLLEGE, THRIKKAKARA

(Affiliated to Mahatma Gandhi University Accredited by NAAC with A+ Grade)

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" PRODUCTION OF CUSO4 NANOPARTICLES VIA COUROPITA GUIANENSIS LEAF EXTRACT FOR ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES"

Dissertation submitted to

MAHATMA GANDHI UNIVERSITY, KOTTAYAM

in partial fulfilment of the requirement for the degree of

BACHELOR OF SCIENCE

Submitted by

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Under the supervision of

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THRIKKAKKARA Date:

CERTIFICATE

This is to certify that the project entitled **" Production of Nanoparticles Via Couroupita guianensis Leaf Extract & its Antimicrobial & Antioxidant Properties"** is a bonafide work done **by NANDANA K DIVAKARAN with Register No: 210021037724** during 2023-24 in partial fulfilment of the requirement for the award of the Bachelor Degree of Science in Zoology of Mahatma Gandhi University, Kottayam.

Head of the Department

Dr. Simi Joseph P

DECLARATION

I, **NANDANA K DIVAKARAN (210021037724),** hereby declare that the dissertation work entitled **"Production of CuSO4 Nanoparticles via Couroupita guianensis Leaf Extract for Antimicrobial and Antioxidant Properties"** submitted for the award of a Bachelor's degree in Bharata Mata College, Thrikkakara, is a partial fulfillment of the requirements. This work was done by me during the period from December 2023 to February 2024 under the supervision and guidance of Dr. Sherin Antony, Assistant Professor, Department of Zoology. I affirm that this thesis is original and has not been submitted for any degree, fellowship, or similar qualification by any other candidate to any university.

Date:

Place: Thrikkakara

Signature:

NANDANA K DIVAKARAN

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ABSTRACT

Couroupita guianensis, known as the cannonball tree, is a deciduous plant in the Lecythidaceae family. It is famous for its round fruits housed in a tough shell, which gives it its name. The fruit's outer skin starts white and turns blue when exposed to air. Despite being edible, people don't consume it much because of its unpleasant smell, unlike its beautiful blossoms. It has been used for treating various illnesses like malaria and inflammation, although its effectiveness isn't well-documented. Green synthesis, using plant extracts, is an eco-friendly alternative to traditional chemical methods. C. guianensis leaves have compounds like flavonoids and terpenoids that offer antiviral, antioxidant, and anti-inflammatory effects. These properties can help with fungal infections, oxidative damage, and microbial issues. The plant may also help combat diseases spread by mosquitoes. Nanoparticles made from the plant have shown potential in medical applications due to their low harm and ability to fight bacteria effectively.

This article discusses to create copper nanoparticles using aqueous extracts derived from Couroupita guianensis Aubl. The process is described as eco-friendly, cost-effective, and efficient. Petals, stems, bark, and leaves were utilized to synthesize the nanoparticles. These plant extracts facilitated the reduction of Cu2+ ions into CuNP and provided stability. . The newly formed nanoparticles displayed significant antibacterial properties against Staphylococcus saprophyticus and Escherichia coli. This antibacterial efficacy indicates the potential of utilizing copper nanoparticles, produced inexpensively using leaves of Couroupita guianensis Aubl .

Keywords:couropita guianesis,CUSO4

INTRODUCTION

Nanoparticles are widely used in various fields such as cosmetics, drug delivery, and pharmaceutical materials to enhance material properties. Nanotoxicology is crucial for safe and sustainable nanotechnology advancement. Nanoscale structures are being organized into superstructures, ensuring nanotechnology's role in multiple technologies. The small size and high surface-to-volume ratio of nanoparticles make them highly appealing. Copper nanoparticles can be synthesized physically, chemically, or biologically, with biosynthesized ones offering improved stability and biocompatibility. Copper nanoparticles are prized for their conductivity, melting point, and cost-effectiveness compared to other metals. They also oxidize to form stable properties. Green nanotechnology is a promising area where functional nanoparticles are produced from iron, zinc, copper, and gold without using harmful chemicals.

The eco-friendly synthesis of nanoparticles is considered an important and cost-efficient process that prioritizes environmental protection. Utilizing natural resources like plant extracts, bacteria, fungi, enzymes, and algae for nanoparticle biosynthesis is a growing field. These resources contain useful compounds that can be used to create nanoparticles, offering advantages like energy efficiency, cost-effectiveness, and environmentally safe chemicals. Nanoparticles produced through biosynthesis have shown promising medicinal properties, including antimicrobial, antioxidant, antimalarial, anti-inflammatory, anti-diabetic, and anticancer effects. In this study, active components from C. guianensis extract were used to produce copper nanostructures for antimicrobial purposes. The advancement and use of nanotechnologies have led to numerous possibilities in research and industry, creating new materials with a wide range of applications across various sectors. Nanoparticles, as a type of nanomaterial, have garnered considerable interest for their diverse uses in different fields.

Image of Couroupita guianensis Aubl flower

image of Couroupita guianensis tree is known by a variety of common names including cannonball tree.

AIM AND OBJECTIVE

AIM

This study aims to use leaf extract from Couroupita guianensis to create copper sulfate nanoparticles and assess their antibacterial and antioxidant qualities.

OBJECTIVE

To Evaluate the effectiveness of the copper sulphate nanoparticles against bacteria. Examine the potential antioxidant properties of the plant extract.

REVIEW OF LITERATURE

The Lecythidaceae family comprises large tropical deciduous trees like the cannonball tree (Couroupita guianensis Aubl.). The active compounds found in various parts of the cannonball tree, such as leaves, stems, flowers, and bark, have potential healing properties. This potential is attributed to essential oils, glycosides, ketosteroids, isatin, indurubin, and phenolic chemicals present in the plant The chemical components of the tree's leaves include hydroxycinnamic acids, rosmarinic acid, and various other substances. Leaf extracts of the cannonball tree show antibacterial and antifungal properties that can help in treating various ailments). The Lecythidaceae family consists of large tropical deciduous trees like the cannonball tree (Couroupita guianensis Aubl.). The medicinal properties of various parts of the plant, such as leaves, stems, flowers, bark, etc., contain bioactive compounds that can be used for healing purposes. These healing properties are attributed to essential oils, glycosides, ketosteroids, isatin, indurubin, and phenolic chemicals found in the plant (Pandurangan et al., 2018).

The leaf extracts of C. guianensis contain phenolic and volatile compounds that exhibit antibacterial and antifungal effects, making them effective in treating various diseases (Elumalai et al., 2012).

Because EO water uses a small amount of salt solution and does not contain extra chemical additives, it has a reduced environmental impact in terms of chemicals (Kim et al., 2000).

Nanoparticles are now widely used in various aspects of our lives, including cosmetics and drug delivery systems. They play a significant role by improving the properties of various materials in therapeutic, biosensor, and pharmaceutical applicationsNithiyavathi et al., 2021, Poovendran et al., 2020, George et al., 2022).Nanoparticles have generated significant interest due to their extremely small size and large surface-to-volume ratio (Theophil Anand et al., 2019, Manjula et al., 2018).

Copper nanoparticles were produced through physical, chemical, and biological methods (EL-Din Hassan et al., 2018).

Copper nanoparticles are increasingly popular because of their high electrical conductivity, elevated melting point, minimal electrochemical migration, and cost-effectiveness compared to metals like silver, gold, platinum, and palladium (Rajesh et al., 2018, Rajeshkumar and Rinitha, 2018, Rehana et al., 2017).

Moreover, copper nanoparticles have the advantage of oxidizing to form stable nanoparticles with consistent chemical and physical properties. The field of green nanotechnology has emerged, focusing on the creation of functional nanoparticles using iron, zinc, copper, and gold without the need for toxic chemicals (Nazar, 2018).

The green synthesis of nanoparticles (NPs) is considered a safer, more cost-effective, and environmentally friendly approach compared to physical and chemical methods [1].(Abdelghany, T.M.; Al-Rajhi, A.M.H.; al Abboud, M.A.; Alawlaqi, M.M.; Ganash Magdah, A.; Helmy, E.A.M.; Mabrouk, A.S.)

Metal NPs like Au, Ag, Zn, and Cu have been successfully synthesized and studied for various medical purposes. Among these, silver (Ag) NPs are commonly produced using plant extracts. Ag NPs are renowned for their distinct features such as excellent thermal and electrical conductivities, enhanced stability, and promising biological effects [2].(Netala, V.R.; Kotakadi, V.S.; Nagam, V.; Bobbu, P.; Ghosh, S.B.; Tartte, V.)

Biologically synthesized Ag NPs are gaining significant attention in biomedical fields, particularly in drug development for infectious diseases, drug delivery systems, diagnostics, and mosquito control [3].(Huy, T.Q.; Huyen, P.T.M.; Le, A.-T.; Tonezzer, M)

Ag NPs ranging from 1 to 100 nm in size, coupled with a large surface area and highly reactive surfaces, are attracting significant interest [4,5,6,7,8].(Elangovan, K.; Elumalai, D.; Anupriya, S.; Shenbhagaraman, R.; Kaleena, P.K.; Murugesan, K.4.Salata, O.5.Shanmugasundaram, T.; Balagurunathan, R.6."Benelli, G.; Govindarajan, M."7."Benelli, G.; Govindarajan, M.8)

In this research, extracts from Lagerstroemia speciosa (L. speciosa) fruits and Couroupita guianensis (C. guianensis) flowers were utilized in the creation of Ag NPs. The plant L. speciosa, commonly known as "Jarul," belongs to the Lythraceae family [9].("Sondi, I.; Salopek-Sondi, B.)

These plant parts contain various phytochemicals like alkaloids, terpenoids, flavonoids, and others [10,11]."Myint, P.P.; Soe, M.T.; Hlaing, H.H","Pareek, A.; Suthar, M.; Rathore, G.S.; Bansal, V")

Moreover, both the leaf and flower extracts from L. speciosa have been extensively examined for the green synthesis of Ag NPs [8] and studied for a range of biological activities, including antibacterial, anti-diabetic, and anti-inflammatory properties [12,13].("Al-Snafi, A.E. ","Sharmin, T.; Rahman, M.; Mohammadi, H.")

Additionally, components of C. guianensis (also known as ayahuma and cannonball tree), a plant from the Lecythidaceae family, have been explored for their potential in anticancer, antifungal, and anti-inflammatory applications. Extracts from these plant parts have been traditionally used to alleviate common cold symptoms, stomach aches, and malaria [14,15].(: "Sumathi, S.; Anuradha, R, "Sheba, L.A.; Anuradha, V.")

Recent findings have demonstrated that leaf and fruit extracts of C. guianensis are effective in the rapid and cost-effective production of Ag NPs and controlling the dengue vector Aedes aegypti [16].

Yet, there has been limited research on the biological properties of Ag NPs synthesized from plant extracts. Therefore, this study aims to investigate the in vitro antibacterial, antioxidant, larvicidal, and cytotoxic properties of Ag NPs produced using extracts from

L. specieosa fruits and C. guianensis flowers. The combination of bioactive compounds from these sources can lead to Ag NPs with diverse biological effects.

MATERIAL AND METHODS

1. Collection of plants and substances

Fresh and healthy leaves, of C. guianensis were gathered from the college premises. They were cleaned, dried in the shade for 5-7 days, and then ground into a fine powder using an electric mixer

2. Preparation of plant extracts

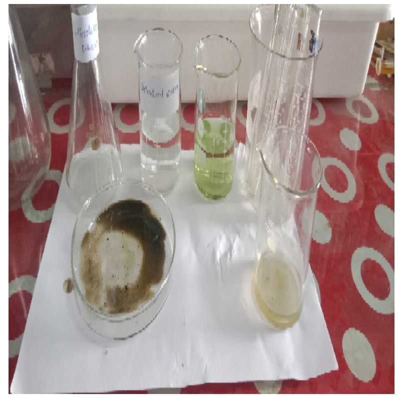
To make the plant extract, 10 grams of powdered leaves, stems, petals, and barks were combined with 100 ml of deionized water and boiled at 60 °C for 30 minutes. The mixture was then filtered using Whatman No.1 paper to eliminate any residue and stored at 4 °C for future use.

3.To synthesize CuNPs, 0.25 g of $CuSO_4$ was dissolved in 50 ml of water. Then, 4 ml of C. guianensis extract was added slowly while stirring for 10 minutes until a dark green color appeared. The mixture was stirred at 60 °C for 1-2 hours, left at room temperature for 12-14 hours, and then centrifuged at 10,000 rpm for 10 minutes. The resulting solid was washed with water and ethanol several times to remove impurities before drying in an oven at 70 °C for 10 hours.

PREPARATIONS OF NANOPARTICLES

A) 

B) 



RESULT AND DISCUSSION

The compound was tested for its ability to inhibit the growth of two bacterial strains, Staphylococcus aureus and Escherichia coli. The results showed varying levels of antimicrobial activity, ranging from moderate to good. The antibacterial activity was assessed using the agar well diffusion technique. The diameter of the inhibition zone was measured after 24 hours of bacterial incubation at 30 °C. A filter paper disc saturated with a sample of the compound was placed on agar medium seeded with the test organism. The clear zone of inhibition surrounding the sample was measured to determine its inhibitory power. The antimicrobial activity of the tested compounds was evaluated against both gram-positive and gram -negative bacteria.

Many studies have shown that nanomaterials have unique characteristics that make them suitable for use in personal care products, cosmetics, medicines, drug delivery systems, and textiles. Green synthesis of nanoparticles offers a more sustainable alternative to traditional methods that involve toxic solvents, chemicals, and harsh reaction conditions like high temperatures and pressure. This environmentally friendly approach allows for the cost-effective and efficient production of nanoparticles with improved stability, effectiveness, purity, and uniformity in size.

E. coli, or Escherichia coli, is a bacteria commonly found in the intestines of humans and animals. While most are harmless, some strains can lead to food poisoning. E. coli is extensively studied in microbiology and genetics due to its quick growth and well-known genetics. Although it can be treated with antibiotics like penicillin, cephalosporins, fluoroquinolones, and aminoglycosides, misuse has led to the rise of antibiotic-resistant strains, presenting challenges in treating infections caused by E. coli.

Antibacterial disc	3.6cm
Ethanol	0.5cm

DMSO	0.4cm
Ethanol + Nanoparticles (100µl)	1.1cm
DMSO + Nanoparticles (100µl)	1.6cm

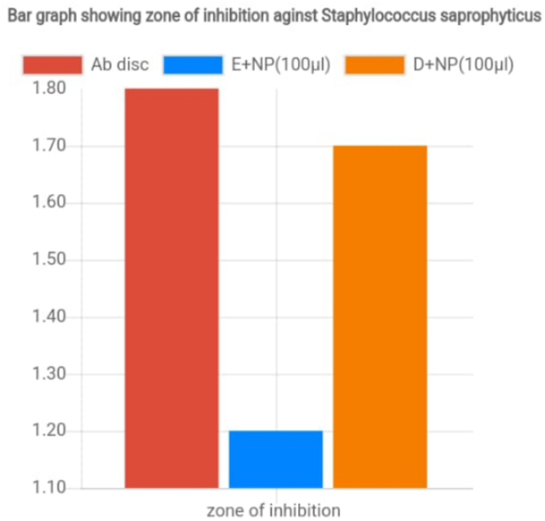
a) Antibacterial effect shown against E.coli

Staphylococcus saprophyticus is a bacteria commonly found in the gastrointestinal and genital tracts of humans and is a frequent cause of UTIs, especially in young sexually active females. It is typically treatable with antibiotics like trimethoprim-sulfamethoxazole, nitrofurantoin, and fluoroquinolones, but resistance can develop. This resistance can present difficulties in effectively treating infections caused by this bacterium.

Antibacterial disc	1.8cm
Ethanol	0.3cm
DMSO	0.2 cm
Ethanol +Nanoparticles (100µl)	1.2cm
DMSO+Nanoparticles (100µl)	1.7cm

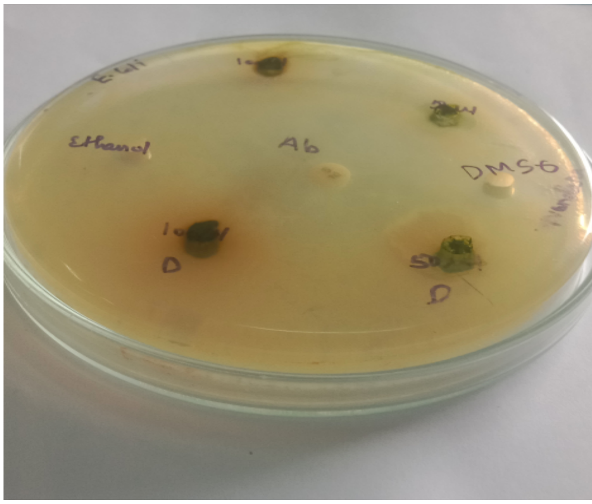
(b) Antibacterial effect shown against Staphylococcus saprophyticus

The study focused on creating CuO nanoparticles using Couroupita guianensis, also known as the cannonball tree, which is an interesting topic of research. Green synthesis techniques are becoming popular due to their eco-friendly nature and potential uses in different fields. CuO nanoparticles made with Couroupita guianensis have shown promising antibacterial and antifungal properties. They have proven effective against a variety of microorganisms, including Escherichia coli, Staphylococcus saprophyticus, and Candida albicans. The CuO nanoparticles also have antioxidant properties, with studies showing they have significant antioxidant activity by scavenging free radicals and preventing oxidative damage. This makes them valuable for potential applications in medicine and cosmetics, where antioxidants are used for protection against diseases and aging.

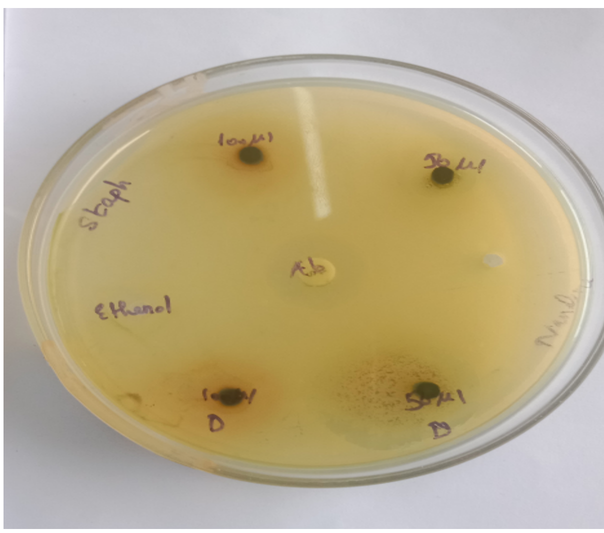


ANTIBACTERIAL ACTIVITY

The tested compound was prepared and evaluatedfor its antibacterial activity against Staphylococcus aureus and Escherichia coli through the diffusionplate method. Results showed a range of moderate togood antimicrobial activity. The bacteria werecultured on Czapek-Dox agar medium and theinhibition zones were measured after 24 hours ofincubation at 30°C. The diameter of the clear zonesurrounding the sample was used to determine theinhibitory power of the compound. Variousreferences were consulted for the examination ofthe antimicrobialactivity against the tested bacteria.



Antbacterial effect shown against Escherichia coli



Antbacterial effect shown against Staphylococcus aureus

CONCLUSION

In the present work, the synthesis of copper nanoparticles was investigated using aqueous extracts of Couroupita Guianensis Aubl leaves as a stabilizer agent. A simple and feasible method is presented in this work to produce copper nanoparticles with desirable functional properties.The characterization of CuNPs were subjected to the antibacterial activity for gram-negative and gram-positive bacterial strains, and exhibited good results for E . coli and Staphylococcus saprophyticus . The CuNPs may be an effective product used for many biomedical applications, which could be a potential agent.

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