BHARATA MATA COLLEGE, THRIKKAKARA

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"GREEN SYNTHESIS OF ZnO NANOPARTICLES USING COUROUPITA GUIANENSIS LEAF EXTRACT AND ITS ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY"

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

Dr. Sherin Antony

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DEPARTMENT OF ZOOLOGY
BHARATA MATA COLLEGE
THRIKKAKKARA **Date:**

CERTIFICATE

This is to certify that the project entitled **“Green Synthesis of ZnO Nanoparticles Using *Couroupita guianensis* Leaf Extract & its Antimicrobial & Antioxidant Activity”** is a bonafide work done by **MARY CANDRIYA PAIVA** with Register No: **210021037721** 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, **MARY CANDRIYA PAIVA (210021037721),** hereby declare that the dissertation work entitled **"Green Synthesis of Zno Nanoparticles Using *Couroupita guianensis* Leaf Extract and its Antimicrobial and Antioxidant Activity"** 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:

MARY CANDRIYA PAIVA

ABSTRACT

Couroupita guianensis, recognized under various vernacular designations such as cannonball tree, represents a deciduous specimen within the Lecythidaceae botanical family, renowned for its spherical fruits encased in a sturdy shell that bestows upon the plant the epithet "cannonball tree". The outer layer of the fruit boasts a white hue, transitioning to a blue shade upon exposure to oxygen, a process facilitated by atmospheric interaction. Despite the edible nature of the fruit, human consumption remains limited due to its off-putting aroma, diverging from the captivating scent emitted by its blossoms. Although there is a dearth of information regarding its effectiveness, it has been used to treat a variety of illnesses including malaria, toothache, stomachache, common cold, tumors, discomfort, and inflammation.The green synthesis approach provides a sustainable option to traditional chemical methods, harnessing plant extracts' reducing and stabilizing properties.

C. guianensis leaf extracts consist of flavonoids, tannins, terpenoids, and alkaloids, known for their therapeutic advantages including antiviral, antifungal, antiprotozoal, antioxidant, and anti-inflammatory properties. The plant can be utilized to address fungal illnesses, oxidative damage, and microbial infections. The plant has the potential to combat mosquito vectors that spread arboviral illnesses. Synthesized ZnO nanoparticles demonstrate a very low hemoglobin release and an excellent bactericidal action against human infections, indicating their applicability for a wide range of nanomedical applications.

This study outlines the synthesis of Zinc oxide nanoparticles using *Couroupita guianensis* leaf extract and evaluates their antimicrobial and antioxidant effectiveness. The synthesized nanoparticles exhibit promising inhibitory effects against a range of pathogenic microorganisms, indicating significant antimicrobial activity. Additionally, the antioxidant potential of ZnO-NPs is evaluated through scavenging assays, highlighting their ability to neutralize free radicals. In conclusion, employing Couroupita guianensis leaf extract for green synthesis offers a sustainable and effective means of producing ZnO-NPs with notable antimicrobial and antioxidant properties. These nanoparticles hold promise for diverse applications in medicine and environmental remediation.

Nevertheless, the antibacterial activity of ZnO nanoparticles synthesized from Cannonball tree leaf extract is not evaluated using gram-negative *E. coli* and gram-positive *Staphylococcus saprophyticus* strains. To ascertain its effects, it is therefore required to examine the Cannonball tree's NP's antibacterial efficacy against the particular pathogens. The *Couroupita guianensis* plant's antioxidant capacity is also examined.

Key words : *Couroupita guianensis*, ZnO nanoparticle, Antibacterial effect, Antioxidant.

REVIEW OF LITERATURE

Couroupita guianensis Aublet (Family: Lecythiadaceae) is a commonly known tree in India, referred to as Naglingam or the Canaball tree. It can be found throughout the country's plains. The flowers of this tree are utilized for the treatment of colds, intestinal gas formation, and stomachaches, as mentioned in the Wealth of India. It is also recognized by other names such as the "Cannonball tree," "Sal tree," or "Ayauma tree." The leaves, blossoms, fruit, and stem of the tree have all been used to treat a variety of ailments because of its many medicinal qualities. This plant holds great importance in traditional veterinary medicine, as it has been utilized commercially for a long time. In recent decades, there have been numerous reports highlighting the plant's anti-inflammatory, anti-ulcer, and anti-cancer activities.The extract from different parts of Couroupita guianensis boasts an array of compounds, including oils, keto steroids, glycosides, couroupitine, indirubin[1], isatin, and phenolic substances, showcasing notable pharmacological activities[2]. Hence, additional research is necessary to identify these pharmacologically active compounds[3,4,5,6]. They hold potential for the creation of innovative drugs to address a range of diseases.Scientists can explore the potential of this tree in treating different illnesses through future research, thus providing an attractive niche for scientific exploration. With nanoscale structures (nanoparticles) in the environment and biomedicine, nanotechnology is fundamental to many important technologies. The literature has examined the synthesis of zinc oxide nanoparticles (ZnONPs) through the utilization of *Couroupita guianensis* leaf extract. Furthermore, the antimicrobial properties of these nanoparticles have been investigated. Diverse investigations have explored the green synthesis of ZnONPs using various plant extracts such as *Pluchea indica*, *Viscum album*, *Punica granatum*, cinnamon, and bay leaves. The studies showed that the synthesized ZnONPs exhibit antimicrobial effects on various bacteria and fungi such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Bacillus subtilis*, *Candida albicans*, and *Cryptococcus neoformans*[7,8,9]. The size of the synthesized ZnONPs varied from 9.968 to 45 nm. In comparison to unprocessed herbal medicinal extracts, the green-synthesized ZnONPs have exhibited superior antibacterial activity. In summary, the literature suggests that the green synthesis of ZnONPs utilizing *Couroupita guianensis* leaf extract holds potential for antimicrobial applications.

The comprehensive literature survey has uncovered that *Couroupita guianensis* is a significant medicinal plant with a wide range of pharmacological effects. The plant exhibits the presence of numerous chemical constituents that contribute to its diverse pharmacological and medicinal properties. *Couroupita guianensis* contains a diverse array of active compounds such as steroids, glycosides, carbohydrates, couroupitone A, couroupitone B, isatin, triterpenoids, eugenol, linoleic acid, nerol, tryptanthrin, linalool, phenolic resin substances, and dyes.[1,2,10,11,17]. In summary, it appears that this plant holds great potential as an intriguing field of study for scientists and holds promise for the betterment of humanity. **(Vivek P. Chavda, 2015)**

In the recent study of cannonball trees grown in Thailand, researchers noted that the size of the cannonball tree hood staminodes was significantly larger than that of the ring stamens. The reason behind this difference is currently unidentified. Currently, it is yet to be determined if genotype-environment interactions could potentially be linked to this phenomenon and further investigation is warranted. It's crucial to investigate whether this phenomenon occurs in cannonball trees from different geographic regions. [12]. Additionally, the pollen from the hood staminodes showed differences from that of the ring stamens[13]. The hood staminodes were sterile and couldn't germinate even with sucrose concentrations that typically promote germination. These discoveries about pollen are consistent with studies on cannonball trees from other regions, indicating that the sizes of male reproductive organs in these trees might not accurately reflect the fertility of their pollen.[16].Moreover, the need for sucrose in pollen germination in vitro seems to remain consistent among trees cultivated in various geographical areas.[14,15]. From a possible evolutionary perspective, the tetrad hood pollen resembled the pollen of certain primitive angiosperms, rather than the ring monad pollen.**(Kiatphaibool Permchalad, Saranya Changbandist and Saowaros Phanomchai,Leupol Punnakanta,2016)**

Whole-plant extracts have diverse benefits, including wound healing and antimicrobial effects. They also inhibit HIV-1 reverse transcriptase, hinting at antiviral potential. Extracts from roots act as antidepressants, while flower extracts are rich in antioxidants. They combat human intestinal worms and mosquito larvae, offering promising solutions. This tropical tree holds untapped remedies, promising vast research opportunities for human welfare[18]. **(Chee Beng Jin, 2013)**

An in vitro culture system has been established for adventitious root cultures of the threatened plant *C. guianensis*, offering an alternative to root exploitation for pharmaceutical industries. Maximum adventitious roots were induced from mature in vitro leaves of 30-day-old seedlings. A half-strength MS semisolid medium with 2.0 mg/L IBA was most suitable for primary adventitious root induction, while liquid half-strength MS medium with 0.5 mg/L IBA optimized biomass production. This is the first report on the influence of exogenous auxins in adventitious root cultures of *Couroupita guianensis Aubl*[19]. **(M Manokari, Mahipal S Shekhawat,2016)**

The chloroform extract showed potent antibiofilm activity against *Pseudomonas aeruginosa* at a concentration of 2 mg/mL, resulting in a 52% inhibition of biofilm formation. Analysis revealed the presence of Indirubin as a major constituent, comprising 0.0918% of the dry weight basis. This suggests potential for drug development programs due to its promising antimicrobial and antibiofilm properties[20]. **(Naif Abdullah Al-Dhabi, Chandrasekar Balachandran, Vikrant Singh Rajput,2012)**

The utilization of *C. guianensis* leaf extract (at a concentration of 10 ml/L) and EO water (with a pH of 5) proved to be highly effective in preventing the deterioration of weight, decay, firmness, and surface color in tomatoes. Additionally, these treatments successfully preserved the levels of titratable acidity, flavonoids, carotenoids, anthocyanins, vitamin C content, and DPPH radical scavenging. Moreover, the shelf life of the tomatoes was extended by more than three days in comparison to untreated fruits. Consequently, these findings suggest that treating harvested ripe tomatoes with either *C. guianensis* leaf extract (10 ml/L) or EO water (pH 5) can significantly reduce postharvest losses and present a promising approach in the field of postharvest management[21]. **(Farzana Islam, Prosanta Kumar Dash and Shimul Das, 2023)**

The study demonstrates the successful biosynthesis of zinc oxide nanoparticles (ZnONPs) using *Pluchea indica* leaf extract, showcasing their antimicrobial and photocatalytic properties. Characterization techniques such as UV-vis spectroscopy, XRD, FT-IR, EDX, and SEM elucidated the nanoparticles' properties, including size, crystallinity, functional groups, elemental composition, and morphology. The antimicrobial efficacy of the ZnONPs against various pathogens indicates their potential for environmental and biomedical applications[22]. **(Abdulaziz A. Al- Askar, Amr H Hashem Nadeem I. Elhussieny, Ebrahim Saied, 2023)**

The research findings on CG demonstrate its significant antioxidant and antibacterial properties, validating its traditional usage. Extracts from various parts of the plant show scavenging activities against different radicals and exhibit antibacterial activity against common pathogens. Further research focusing on the mechanism of action, isolation of active compounds, pharmacokinetics, and toxicological evaluation is needed to fully explore CG's potential as a source of drugs[23].**(Lawrence Anna Sheba,2ID, Venkatraman Anuradha, 2020)**

The synthesis of ZnO nanoparticles using cinnamon and bay leaf extracts proves to be both eco-friendly and economical. The fact that these nanoparticles show effectiveness against certain bacterial strains opens up exciting possibilities for their use in biomedicine[24]. **(Nedal Ali Hussain, 2023)**

The eco-friendly and cost effective method of synthesizing ZnO nanoparticles through plant mediation is a driving force behind the demonstration of the bio-reduction of ZnO nanoparticles using aqueous extracts derived from various parts of *C. guianensis*, such as the leaf, stem, flower petals, and barks. The confirmation of the presence of ZnO nanoparticles in the reaction mixture was achieved through the utilization of UV-Vis absorption spectroscopy. This particular approach rooted in green chemistry possesses the potential for easy scalability and rapidity[25]. **(M.Manokari and Mahipal S. Shekhawat, 2016)**

A GC-MS analysis was performed on methanolic extracts of *V. album*. The analysis demonstrated that the plant contains an abundance of botanicals and volatile compounds that have potential applications in the field of biomedicine. Successfully achieving the synthesis of nano-zinc oxide was accomplished through a green method utilizing both aqueous and methanolic [29]extracts of *V. album*. The method's efficacy was verified through diverse characterization techniques. X-ray diffractograms revealed that methanolic extracts produced smaller particle sizes, resulting in broader diffraction peaks and modifications in peak positions and intensities, in contrast to larger particles synthesized using aqueous extracts.[28]. A comparative study was conducted to examine the antimicrobial activity and antioxidant potential of the synthesized ZnONPs and their source extracts. The findings suggested that the green-synthesized ZnONPs have the potential to be utilized as nanomedicine for treating microbial infections.[26] **(Ishtiaq, M.; Maqbool, M.; Ahmed, M.; Hussain, I.;Mushtaq, W.; Hussain, T).** The aqueous extracts of the ZnONPs exhibited greater efficacy in inhibiting bacterial growth[27]. Both the aqueous and methanolic extract-mediated green ZnONPs displayed notable antioxidant[30] activity, with the methanolic extracts demonstrating superior effectiveness. Given the challenge of antimicrobial resistance, we propose utilizing green-synthesized nanoparticles as alternative options to antibiotic drugs.[31].**(Muhammad Waqas Mazhar, Ryan Casini and Hosam O. Elansary, 2023)**

ZnO nanoparticles were synthesized utilizing an aqueous extract of *P. granatum* peels to facilitate reduction and capping. The physicochemical characterization involved UV-Vis spectroscopy[31], FT-IR, XRD, SAED, TEM, and SEM-EDX techniques.[31]. The synthesized nanoparticles displayed antimicrobial and catalytic properties, notably targeting Gram-positive bacteria, Gram-negative bacteria, and unicellular fungi, exhibiting MIC values ranging from 12.5 to 6.25 µg mL−1[32]. Furthermore, they demonstrated significant catalytic efficacy in the elimination of MB dye under UV irradiation, achieving a peak removal rate of 93.4% at a concentration of 20 µg mL−1 after 210 minutes of exposure[31]. The stability of the nano-catalyst was validated across five successive cycles, exhibiting only a minor decline in performance. These results underscore the potential of ZnO nanoparticles synthesized via *P. granatum*[33] peel extract as environmentally friendly catalysts with considerable prospects in biomedical and biotechnological domains.**(Amr Fouda, Ebrahim Saied, Ahmed M. Eid and Saad El-Din Hassan ,2023)**

We've successfully synthesized ZnO nanoparticles (NPs) using a cost-effective and eco-friendly method involving *Cocos nucifera* leaf extract, resulting in NPs with an average diameter of 16.6 nm. Various characterization techniques such as[34] UV–Vis spectroscopy, XRD, FTIR, EDX, and SEM were employed, confirming the identity and properties of the NPs. UV–Vis spectroscopy revealed absorption maxima at 370 nm, while XRD analysis confirmed the hexagonal wurtzite structure of the ZnO NPs. FTIR analysis[34] indicated the presence of organic functional groups, crucial for NP stabilization[36]. EDX analysis supported the elemental composition of the NPs. Antimicrobial tests exhibited potent activity against pathogens, while the NPs demonstrated significant photocatalytic and moderate antioxidant[35] properties. These findings suggest potential applications in biomedical, pharmaceutical, and photocatalytic[37]processes.**(Farjana Rahman, Md Abdul, Abu Bakar Siddique, Muhammad Shahriar Bashar, 2022)**

The aim of this investigation is to compare extracts from *Couroupita guianensis* leaves, flowers, and fruits, highlighting variations in their physical characteristics[44] and phytochemical content. Ethyl acetate and chloroform extracts exhibited higher phytochemical[38-41]prevalence and demonstrated effective antioxidant and antibacterial properties, unlike water extracts. TLC analysis[43] supported these findings, showing multiple bands in flower and fruit extracts. Moreover, these extracts were tested for their ability to synthesize silver nanoparticles[44], with flower extracts showing superior performance and significant antimicrobial activity. The synthesized nanoparticles had spherical shapes[45,46] with sizes ranging from 15-57nm. This study underscores the variability in phytochemical composition and bioactivities[42] among different plant components, suggesting their potential use in medical and industrial applications. **(Prakash Pandurangan, Madhumitha Sahadeven, Swetha Sunkar, Sai Krishna Nerella Mohana Dhana, 2017)**

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INTRODUCTION

Ayurveda has been practiced since the second century BC. The ancient Hindu philosophical traditions Vaisheshika and Nyaya, known for their reasoning, established the groundwork for Ayurveda. It was found during the same time as the schools of Nyaya and Vaisheshika developed and it is also connected to the manifestation framework, also referred to as Samkhya. One of the oldest traditional medical systems (TMSs) that is widely used is Ayurveda. There is still much to learn about this conventional medical system's historic wisdom. The convergence of extensive information from various traditional medical systems holds potential to pave new paths for herbal drug research. Among the hurdles hindering the development of plant-based medications, a significant barrier is the insufficient understanding of the disparities and commonalities among the theoretical doctrines of these systems.[47] This would enable aspiring academics, researchers and practitioners to better understand conventional medical systems, deepen their shared experiences and get over obstacles to their widespread acceptance and harmonization. The goal of Ayurveda, the oldest medical science, is to help people live long, healthy, balanced lives through a holistic approach to health. The Sanskrit words ayus, which means life or longevity, and veda, which means knowledge[48,49], are the source of the name Ayurveda. Ayurveda's fundamental principle revolves around maintaining balance within the body, mind, and consciousness through proper nutrition, lifestyle choices, hydration, and herbal remedies, aimed at both preventing and managing diseases.[49].

The deciduous tree Couroupita guianensis[52] belongs to the family Lecythidaceae of flowering plants. It is also referred to by several common names, such as cannonball tree. It is native to the tropical forests of Central and South America[50], and because of its enormous, brownish-grey, aromatic fruits and fragrant blossoms, it is planted in many other tropical regions of the world.Many sections of Couroupita guianensis have potential medical benefits, and the tree is significant in South and Southeast Asian culture and religion.Since the British introduced the cannonball tree to the island in 1881, it has been mistakenly identified as the Sal tree (Shorea robusta) in Sri Lanka and India, leading to its popular use as a feature in Buddhist temples.The French botanist Jean Baptiste Christophore Fusée Aublet gave the tree the name Couroupita guianensis in 1775. The huge deciduous evergreen tree Couroupita guianensis can reach a height of 20 meters. The leaves are oblong-obovate, alternating, hairy on the veins below, whole to slightly serrated, and up to 20 cm long. The enormous branches and trunk give rise to a racemose inflorescence. Stamens are carried on an overhanging androphore, and the flowers are aromatic, reddish-yellow in color. The fruit, which is a large, reddish-brown globose fruit, grows to a size of 15 to 24 cm. It contains a woody capsule that holds 200–300 seeds each.[51]. There have been reports of volatile oils, keto-steroids, glycosides, couroupitone, indirubcin, isatin, and phenolic compounds present in different parts of the tree[1,2,10,11,17].

Planting *Couroupita guianensis* serves two purposes: as a botanical specimen for its intriguing fruit, and as an ornamental for its fragrant, brilliant blooms. Although the fruit is edible, people rarely eat it because it might have a disagreeable scent in contrast to its highly fragrant blooms. Pigs and other domesticated poultry are fed on it. Traditional medicine has made use of plant parts. The Cannonball Tree has analgesic, antifungal, antibiotic, and antiseptic properties. Colds and stomachaches are treated by utilizing trees. South American shamans have even utilized tree pieces to treat malaria; leaf juice is used to heal skin conditions. The fruit's inside can heal wounds, and the young leaves relieve toothaches. The Couroupita guianensis, or cannonball tree, serves multifaceted purposes across cultural, ornamental, and traditional domains. Esteemed for its distinctive flowers, the tree graces ornamental gardens and holds religious significance in various traditions. Traditional medicine systems utilize its bark and leaves, attributing potential medicinal properties. While not a primary source of timber, its wood finds localized applications. The tree's expansive canopy makes it desirable for landscaping and shade in public spaces. Additionally, Couroupita guianensis exhibits aromatic qualities, potentially contributing to essential oil production. Umachigi et al. investigated the healing effects of an ethanolic extract of the entire Couroupita guianensis plant, encompassing its barks, leaves, flowers, and fruits, on excision and incision wound models[53]. The anthelmintic activity of flower extracts in ethanolic, acetone, and chloroform was reported by Rajamanickam et al. in Pheretima posthuma. Worm motility assay, which measured the worms' times of paralysis and death, was used to evaluate the activity. It was discovered that the acetone and chloroform extracts were less effective than the alcoholic extract, and that the activity was on par with that of the common medication piperazine citrate[54]. However, conservation is imperative, given its near-threatened status due to habitat loss. Overall, the tree intertwines aesthetic appeal, cultural relevance, and limited practical applications[55].

Nanotechnology involves crafting, producing, and utilizing structures, devices, and systems by precisely manipulating atoms and molecules at the nanoscale, typically measuring 100 nanometers or less. While natural occurrences exhibit nanometer-scale dimensions and accidental utilization of nanostructures has been prevalent in various technologies, intentional manipulation for specific purposes has become achievable only in recent times[56].Nanotechnology introduces a realm of applications featuring novel materials with distinct properties and unforeseen outcomes compared to their larger counterparts. This is primarily due to the remarkably high surface-to-volume ratio of nanoparticles and the emergence of phenomena observable only at tiny scales. The societal impact of nanotechnology applications holds substantial promise and potential benefits[56]. Aside from the industrial sectors that have already embraced it, including the information and communications industry, food, energy, and several pharmaceutical and medical items also use nanotechnology. Additionally, nanomaterials might present fresh possibilities for lowering pollution levels in the environment. The introduction of novel materials through nanotechnology brings forth potential health hazards. While human defense mechanisms have adapted to various environmental pollutants, the recent exposure to synthetic nanoparticles with unique properties poses new challenges. There's concern that natural defense mechanisms, such as the immune and inflammatory systems, may struggle to effectively combat these nanoparticles. Additionally, nanoparticles have the capacity to disperse widely and persist in the environment, thus impacting it significantly[56].

Plant extract refers to a substance derived from plants that possesses one or more biological functions. This substance has the ability to enhance the production performance of livestock and poultry, improve the quality of livestock products, boost immunity, prevent certain animal diseases, and perform other functions. Research has indicated that the incorporation of plant extracts into animal diets offers numerous biological benefits. These benefits include antioxidative properties, the ability to scavenge excess free radicals within the body, relief from oxidative stress, increased secretion of endogenous enzymes in the digestive tract, reduction in chyme viscosity, improved nutrient utilization, and enhanced production performance. Furthermore, the addition of plant extracts such as saponins and tannins to ruminant diets can contribute to the reduction of rumen methane production and improved nutrient utilization. Plant extracts have been utilized for millennia due to their elevated levels of bioactive components, which are accountable for specific physiological actions within the human body. An abundance of phytochemicals can be found in consumable fruits, flowers, seeds, and leaves, all of which are associated with advantageous effects on health, such as antioxidative, anti-inflammatory, anticancer, hypoglycemic, antiobesity properties, as well as substances that offer protection to the neurological, hepatic, cardiovascular, and gastrointestinal systems. The chemical constituents present within these extracts possess physiological functions within the plants themselves, thus exhibiting remarkable compatibility with the human body. Utilizing plant-based products for nanomaterial creation offers a solution to mitigate the adverse effects linked to conventional synthesis methods reliant on hazardous chemicals. This approach integrates the properties of nanomaterials with those inherent in plant extracts and essential oils, presenting a more environmentally friendly and sustainable alternative[57]. The utilization of plants for nanoparticle synthesis is attractive due to their economical cultivation, rapid production, safety, and scalability. Plants have long been recognized for their ability to reduce metal ions both on their surfaces and in distant organs[59,60] and tissues. Post-harvesting, metals accumulated by plants can be retrieved using sintering and smelting methods. Notably, metals tend to deposit in the form of nanoparticles during the bioaccumulation process in plants, making whole plants suitable for nanoparticle generation. However, industrial application faces limitations. Nanoparticle size and shape vary based on their localization within the plant, influenced by metal ion concentration in tissues and nanoparticle movement. This heterogeneity may hinder precise customization required for certain applications, highlighting challenges in tailoring whole plant-synthesized nanoparticles to meet market demands[58]. Additionally, the extraction, isolation, and purification of nanoparticles from plant material pose significant challenges and result in low recovery rates. In response, in vitro approaches have gained traction in recent years, utilizing plant extracts for the bioreduction of metal ions to form nanoparticles. These methods provide greater flexibility in controlling nanoparticle size and shape, along with simplified purification processes. Crucially, compared to whole plant synthesis, in vitro methods offer faster reaction rates, as they occur almost instantaneously without the time delay required for metal ion uptake and diffusion throughout the plant[59,60].

Nanoparticles are polymeric particles, either natural or artificial, that possess a spherical shape. Their size varies between 10 and 500 nm. Due to their distinctive shape and the high ratio of surface area to volume, these particles offer a broad range of potential applications (Berry & Curtis, 2003). The field of nanoparticle technology is experiencing rapid advancement, leading to the development of innovative and effective treatments for various diseases (Emerich & Thanos, 2003), including neurodegenerative conditions like Alzheimer's and Parkinson's diseases. It is important to acknowledge that future applications of nanoparticles, along with their eventual commercialization, present both human health and environmental risks. Therefore, a thorough assessment of the health and environmental impacts of nanoparticles is imperative in the early stages. Because of their special qualities, metal nanoparticles are becoming more and more important. According to reports, nanoparticles can be used as efficient delivery systems for a variety of compounds, including xenobiotics, insulin, viral antigens, and hemoglobin. The administration of drugs infrastructure, engineering of tissues, therapeutics, bioanalytical diagnostics, and innovative therapies, particularly for cancer, are important domains for nanoparticle applications in medicine[61].

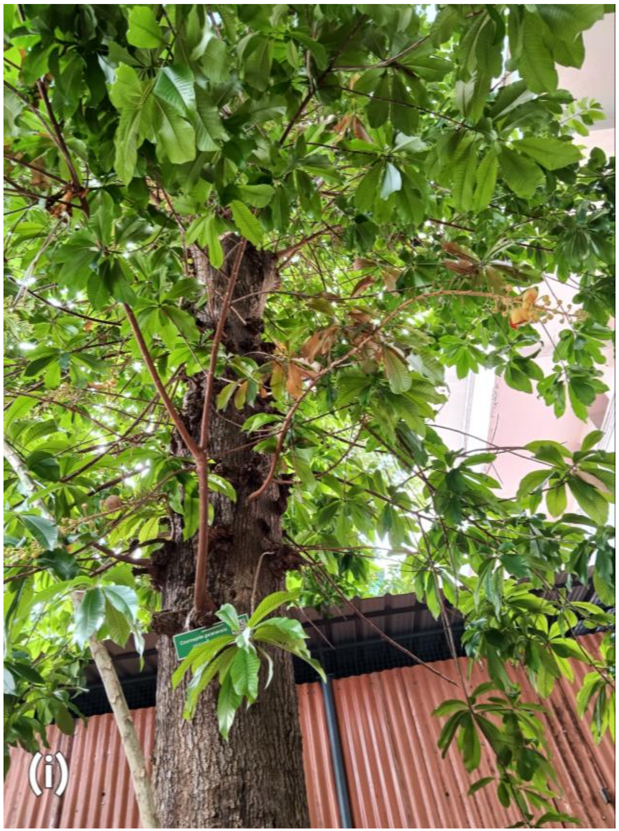
Antioxidants are substances that impede oxidation, a chemical process capable of generating free radicals, typically through autoxidation[62]. Autoxidation results in the deterioration of organic compounds, including living matter. The antioxidant potential of phenols[63,64] found in plant extracts is notably effective even at low concentrations. In humans, this capacity is linked to the prevention of cardiovascular disease [65,66,67] and cancer. Harsh environmental conditions, such as extreme temperatures, drought, heavy metals, nutrient deficiencies, and high salinity, trigger elevated levels of reactive oxygen species (ROS) in plants, leading to oxidative stress. To combat this, cells employ a sophisticated antioxidant system comprising enzymatic and non-enzymatic components. Non-enzymatic molecules within this system exert various actions, including enzyme inhibition, chelation of trace elements involved in free radical production, uptake and activation of reactive species, and reinforcement of protection through other antioxidant defenses[68]. Notably, phenolic compounds derived from secondary metabolism play a pivotal role in mitigating oxidative stress. Several in vitro methods are available for quantifying antioxidant activity, emphasizing the importance of selecting the appropriate method to identify species with the highest antioxidant capacity. Moreover, phenolic compounds not only act as antioxidants by donating hydrogen or electrons but also serve as stable radical intermediates. When consumed as part of the diet, phenolic compounds exert protective effects on humans. Overall, the antioxidant capacity of phenols in plant extracts, effective even at low concentrations, is associated with mitigating cardiovascular disease and cancer in humans. Consequently, research aimed at assessing the antioxidant activity of extracts from different plant species could enhance our understanding of their potential as sources of novel antioxidant compounds[64].

This study explores the eco-friendly synthesis of ZnO nanoparticles using Couroupita guianensis leaf extract, emphasizing the sustainable approach of green synthesis. The synthesized nanoparticles are characterized, and their antimicrobial properties are investigated, revealing potential applications in combating microbial infections. The use of plant extracts in nanoparticle synthesis aligns with the growing interest in environmentally benign methods, contributing to the development of sustainable nanotechnology. Also the method proposed was utilized to ascertain the antioxidant activity of the plant extracts against DPPH.

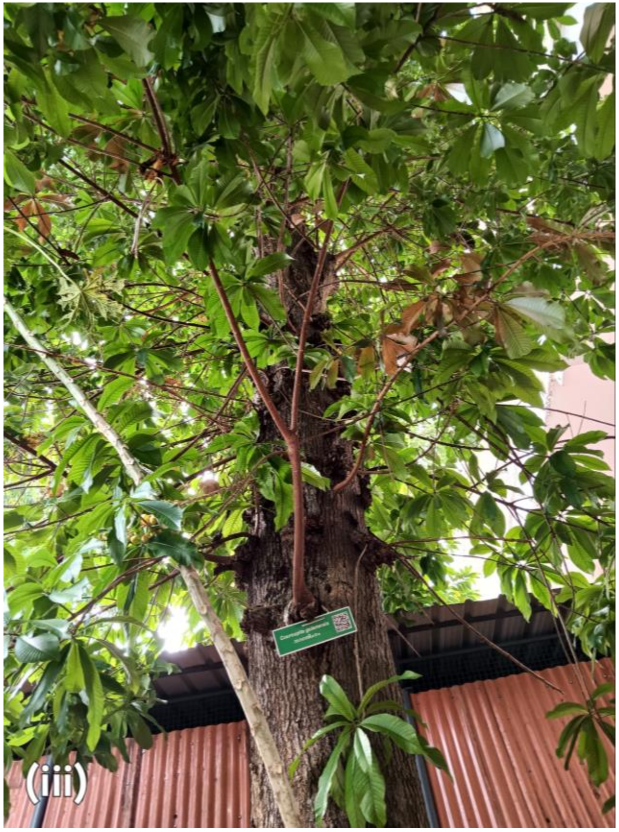
MATERIALS AND METHODS

(i) Plant Material Collection:

Fresh leaves of *Couroupita guianensis* were gathered from the premises of Bharata Mata College, Thrikkakara(Figure i-iii).


(i)


(ii)


(iii)


(iv)

The fresh leaves of *Couroupita guianensis* were collected (Image 3), ensuring they are clean and free from any contaminants(Figure v)**.**

(ii) Leaf Extract Preparation:

The leaves undergo an initial cleansing with distilled water to eliminate any dirt or impurities.Then, 100 gram of fresh leaves are finely ground into a paste (Figure vi)using a blender, yielding 280 gram of paste. Juice is extracted by boiling the paste for 10 minutes in 750 ml of distilled water. After boiling, the resulting extract (620 ml) is cooled to room temperature(Figure vii, viii).

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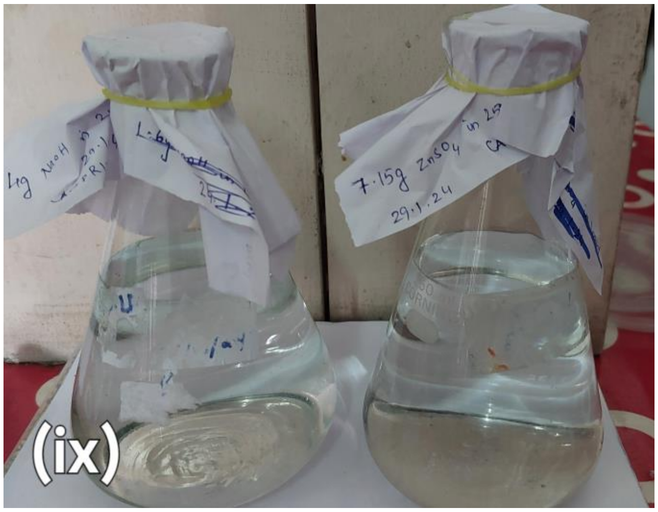
(iii) Preparation of Zinc Oxide Solution:

Zinc sulfate solution at 0.1 M concentration and sodium hydroxide solution at 0.4 N concentration were employed in the fabrication of zinc oxide nanoparticles using the aqueous extract derived from *Couroupita guianensis* leaves(Figure ix).



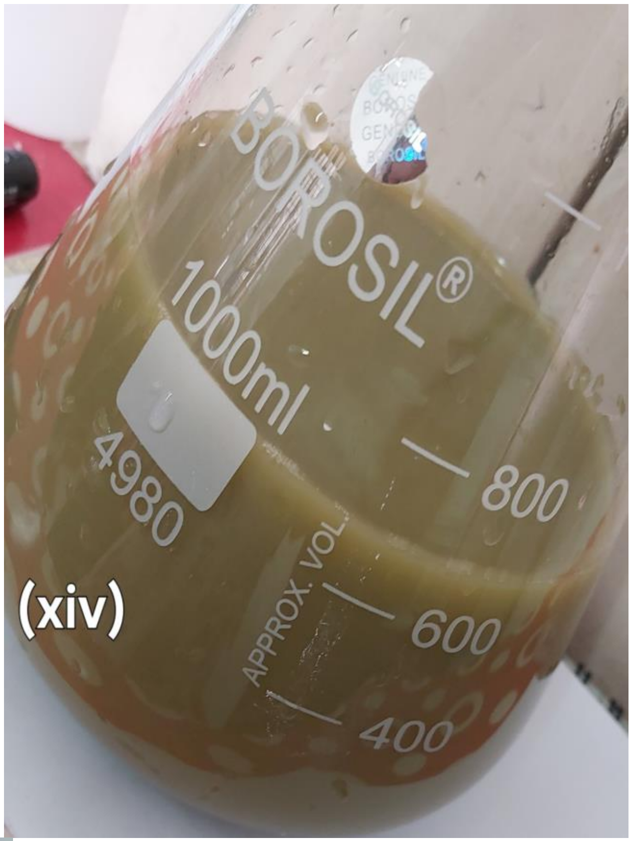
Zinc oxide (ZnO) nanoparticles were synthesized by combining Zinc Sulphate (ZnSO4) and Sodium Hydroxide(NaOH) as precursors. 100 ml of each ZnSO4 and NaOH were mixed, resulting in the formation of a colloidal solution(Figure x).

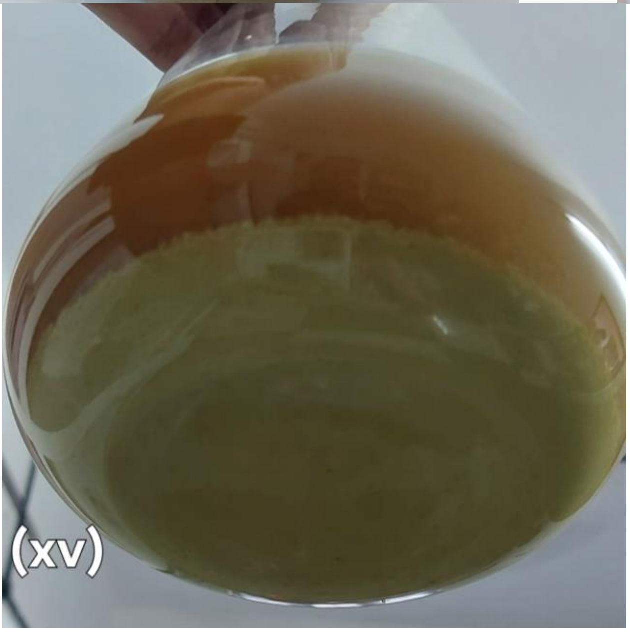
(iv) Synthesis of ZnO Nanoparticles:

100 ml of leaf extract was added to the colloid and incubated in a water bath at 40°C for 20 minutes(Figure xi,xii), resulting in the precipitation of ZnO nanoparticles. The phytochemicals within the leaf extract serve as both reducing and capping agents, facilitating the formation of ZnONPs.

Following this, the mixture was allowed to cool to room temperature for 1 hour (Figure xiii-xix) before undergoing centrifugation at 5000 rpm for 5 minutes to partition the pellet and supernatant. The resultant pellet was transferred onto a watch glass and dried in a hot air oven, yielding a weight of 1.02g. This dried pellet served as the material for subsequent experiments.


(xiii)


(xiv)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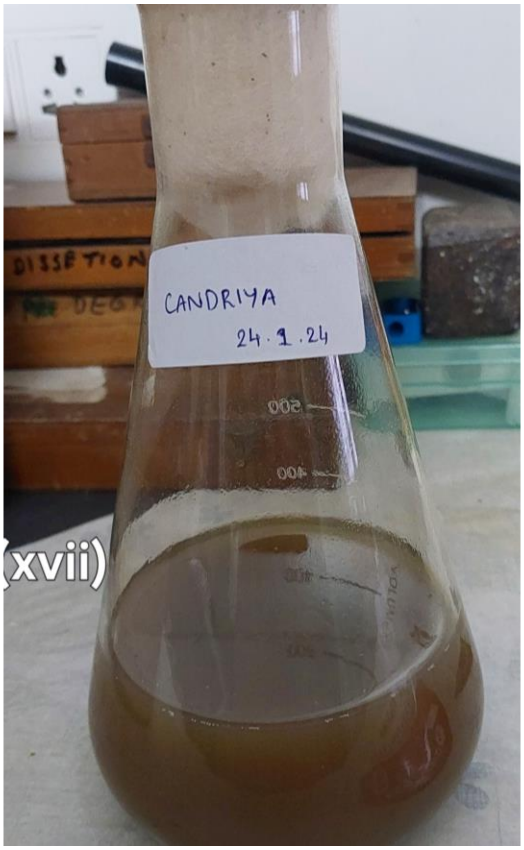
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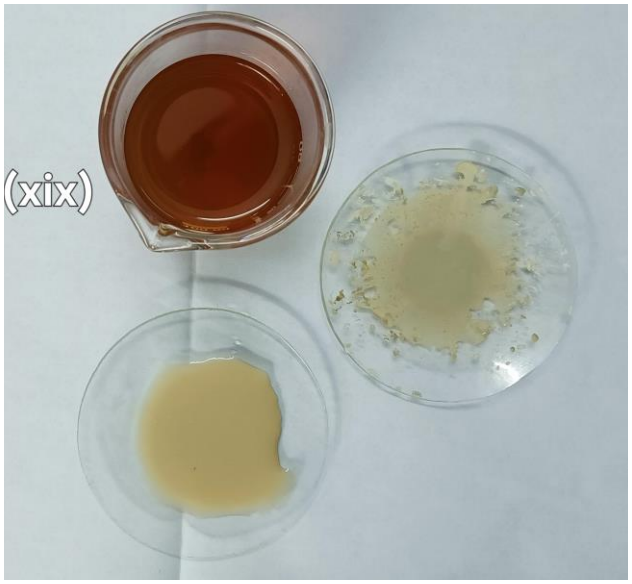
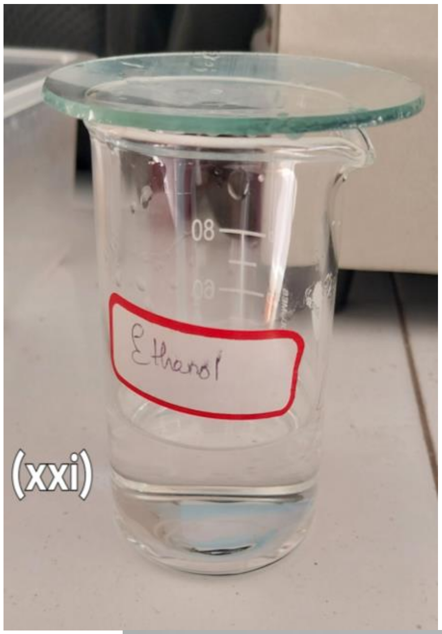
(xviii)


(xix)

(v) Antimicrobial Assay:

We tested the antibacterial activity of ZnO nanoparticles against E. coli and Staphylococcus saprophyticus. Firstly, 0.196g(~0.2g) of ZnO nanoparticles were mixed with 2ml of ethanol to form the 1st concentration (100µl). Next, 1 ml of this solution was transferred to a test tube, to which 1 ml of ethanol was added, yielding the second concentration (50µl). We prepared agar solution and autoclaved the equipment for the antimicrobial assay. The agar solution was poured into petri plates to serve as a growth medium for bacteria. E. coli and Staphylococcus saprophyticus were swabbed onto separate petri dishes, and the prepared concentrations (100µl & 50µl) were pipetted onto the wells using a micropipette. Ethanol was included as a control to assess its effect on bacteria, and a S10 (Streptomycin 10mmg) antibacterial disc was placed in the center. The plates were then incubated for 24 hours in an incubator. Zones of inhibition appeared around each well, and the diameters of these zones were measured(Figure xx-xxviii).


(xx)

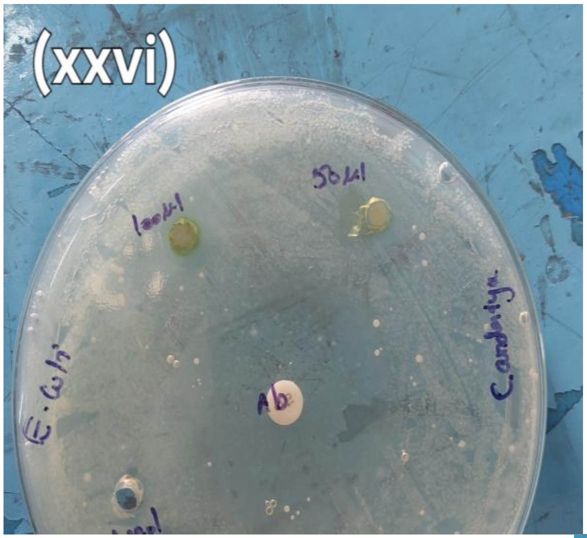

(xxi)

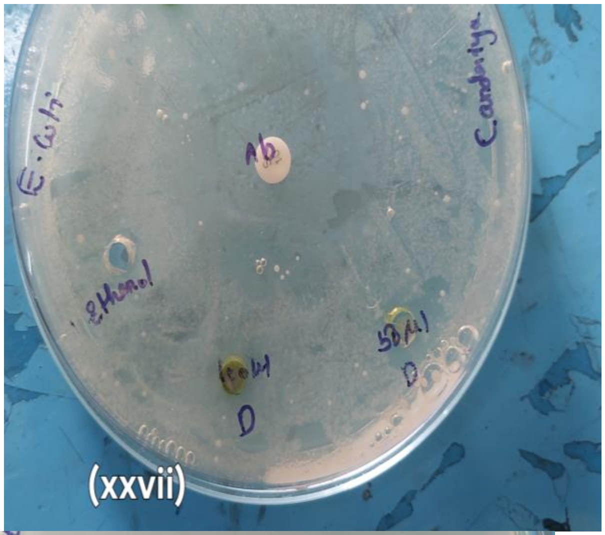

(xxii)

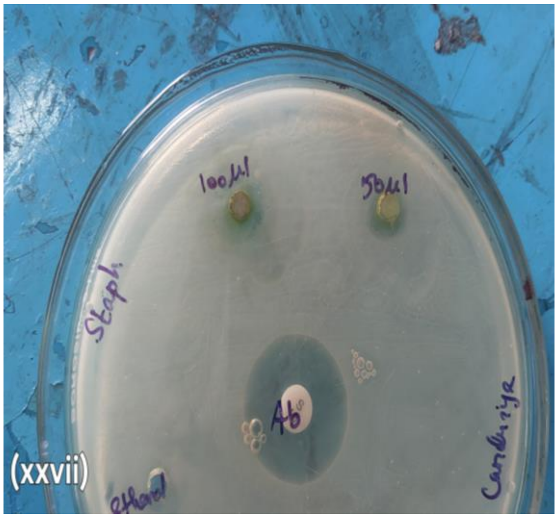

(xxiii)

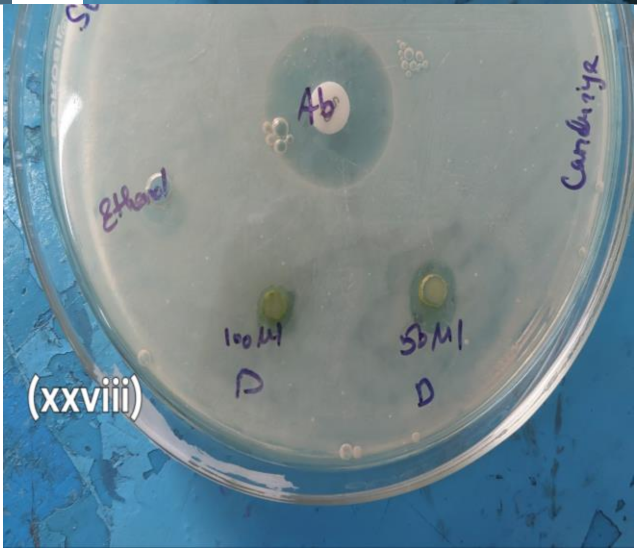

(xxiv)


(xxv)







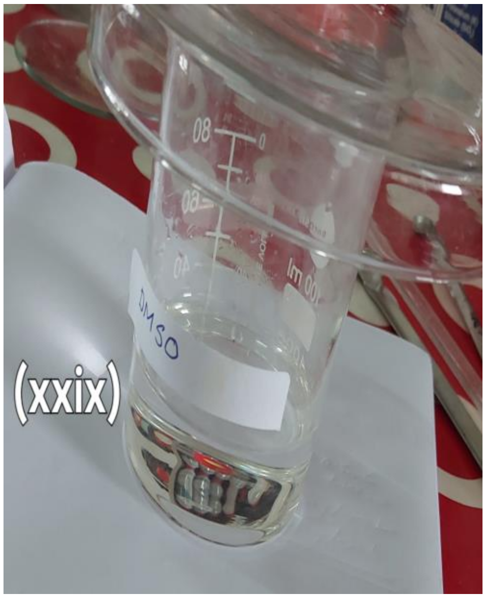


(iv)Antioxidant Assay:

To assess its antioxidant effect, 0.2g of ZnO nanoparticles was mixed with 2ml of DMSO. Three test tubes were prepared: the first contained 3ml of DPPH as a control, the second contained 3ml of DPPH along with ascorbic acid (Vitamin C), and the third contained 3ml of DPPH along with the diluted solution prepared(Figure xxix-xxi). The test tubes were then incubated in the dark for 30 minutes. Subsequently, readings on the spectrophotometer were recorded, yielding the following values:

- DPPH (control) = 1.046
- DPPH + Ascorbic acid = 0.043

- DPPH + Solution = 0.8


(xxix)


(xxx)

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(xxxi)

RESULTS AND DISCUSSION

Zinc oxide possesses appeal for a variety of applications, rendering it a considerable element within numerous industries. With the escalating environmental apprehensions, there has been a rise in the adoption of eco-friendly manufacturing approaches. The literature indicates a surge in interest towards utilizing biological processes for the synthesis of metal nanoparticles and metal oxides at the nanoscale. This methodology has been labeled as "green synthesis." Unlike the prevalent physicochemical techniques employed in industries, this approach showcases reduced adverse ramifications. The complexities associated with the derivation of biological extracts present a hindrance to fully grasping the synthesis procedures and formation mechanisms. Therefore, the examination titled "*Green synthesis of ZnO nanoparticles: a critical review"* delves into various biological substrates and methodologies that could be utilized for the eco-conscious production of zinc oxide nanoparticles[69].**(Archana Harpal and Preeti Pandy, 2023).** The article titled "Synthesis and characterization of CuO nanoparticles utilizing Couroupita guianensis extract for antimicrobial applications" delves into a novel approach for the synthesis of copper nanoparticles using aqueous extracts derived from *Couroupita guianensis*. This method is characterized by its environmentally friendly nature, cost-effectiveness, and efficiency in nanoparticle production. Various parts of the plant such as petals, stems, bark, and leaves were utilized in the synthesis process. The newly synthesized nanoparticles displayed significant antibacterial properties against *Bacillus Subtilis* and *Escherichia coli.* Moreover, the antimicrobial efficacy of these nanoparticles highlights their potential as a cost-effective solution for sustainable applications utilizing *Couroupita guianensis Aubl* extract[70]. **(S. Logambal, C. Maheswari, S. Chandrasekar, T. Thilagavathi, C. Inmozhi, 2022)**

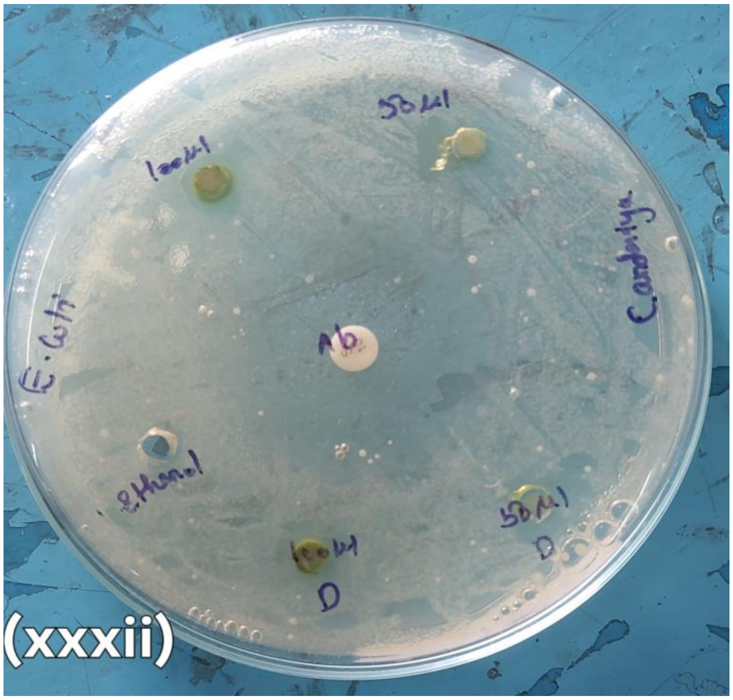
Numerous findings have been documented as a result of the varied physicochemical characteristics of nanomaterials, allowing their integration into personal care items, cosmetics, medications, drug-delivery systems, and textiles. The emergence of green synthesis of nanoparticles (NPs) offers an alternative to traditional physical and chemical techniques, circumventing the use of toxic solvents, chemicals as reducing and capping agents, and harsh reaction conditions like temperature and pressure. This approach enables the synthesis of NPs in a cost-efficient and environmentally friendly manner[71]. Moreover, nanoparticles (NPs) produced through green methodologies exhibit enhanced stability, efficiency, purity, and uniformity in particle size. The scientific community has displayed considerable interest in the green synthesis of nanoparticles (NPs) such as silver, gold, zinc oxide, silicon dioxide, and titanium dioxide. These NPs have exhibited effectiveness as antioxidants and anti-inflammatory agents, attributed to the presence of phytochemicals, biomolecules, and enzymes in biomasses.

These components directly contribute to enhancing antioxidant and anti-inflammatory properties.[71]**(Samudrika Aththanayaka, Gobika Thiripuranathar, and Sagarika Ekanayake,2023).**The analysis of phytochemicals in various extracts of the C. guianensis plant revealed the presence of alkaloids, phenolics, flavonoids, saponins, rutin, quercetin, kaempferol, luteolin, ursolic acid, hopanes, indirubin, isatin, sterols, and fernesol. These compounds played a vital role in the efficient conversion of Zinc Nitrate to ZnO nanoparticles[72].**(Shekhawat et al., 2012).**

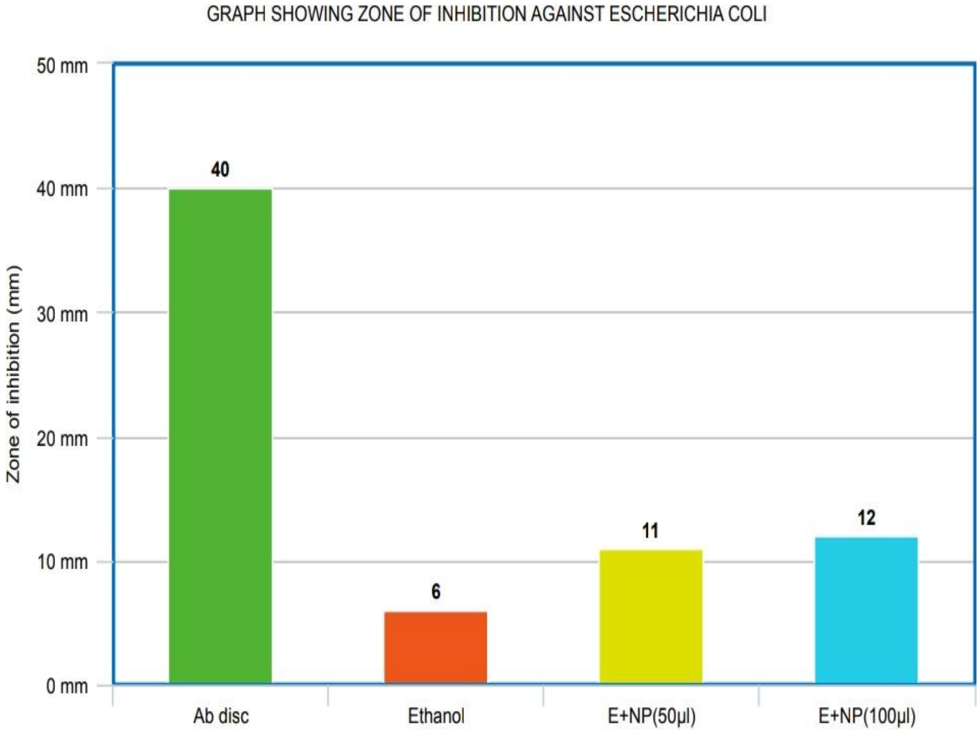
Escherichia coli, or *E. coli,* is a bacteria naturally occurring in the intestines of humans and animals[75]. While many strains are harmless, certain types can lead to food poisoning and other illnesses. E. coli is extensively studied in microbiology and genetics due to its rapid growth and well-understood genetic characteristics due to its rapid growth and well-understood genetics[73]. *E. coli* is susceptible to various antimicrobial agents, including antibiotics such as penicillin, cephalosporins, fluoroquinolones, and aminoglycosides.kMisuse and overuse of antibiotics have resulted in the emergence of antibiotic-resistant strains of *E. coli,* presenting significant challenges in treating infections caused by these bacteria.[74] Antibacterial efficacy against *E.coli* was tested and its zone of inhibition is mentioned below(Table 1;figure xxxii; bar graph 1).

Antibacterial disc	40mm
Ethanol	6mm
Ethanol + Nanoparticle (50µl)	11mm
Ethanol +Nanoparticle (100µl)	12mm

Table 1 : Antibacterial effect shown against *E.coli*



(xxxii)

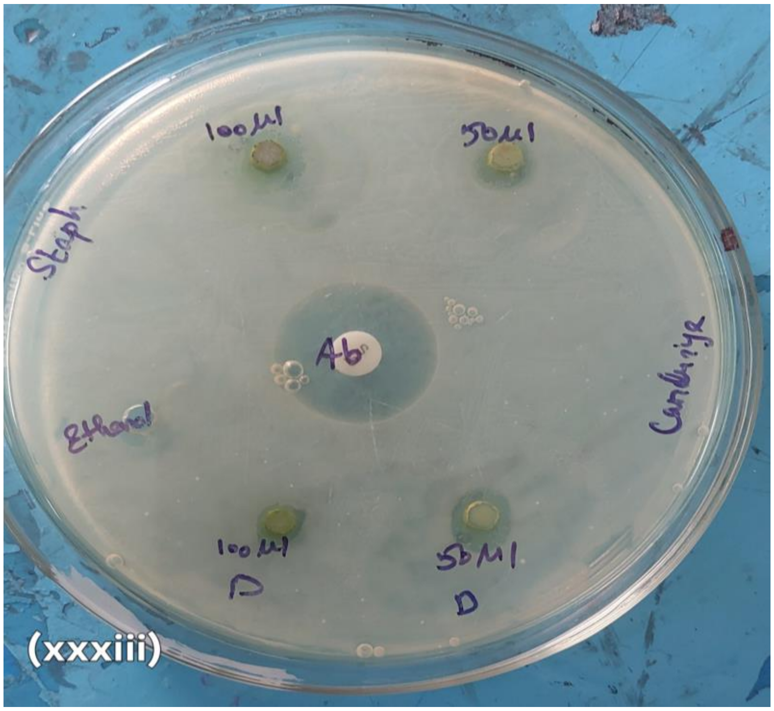


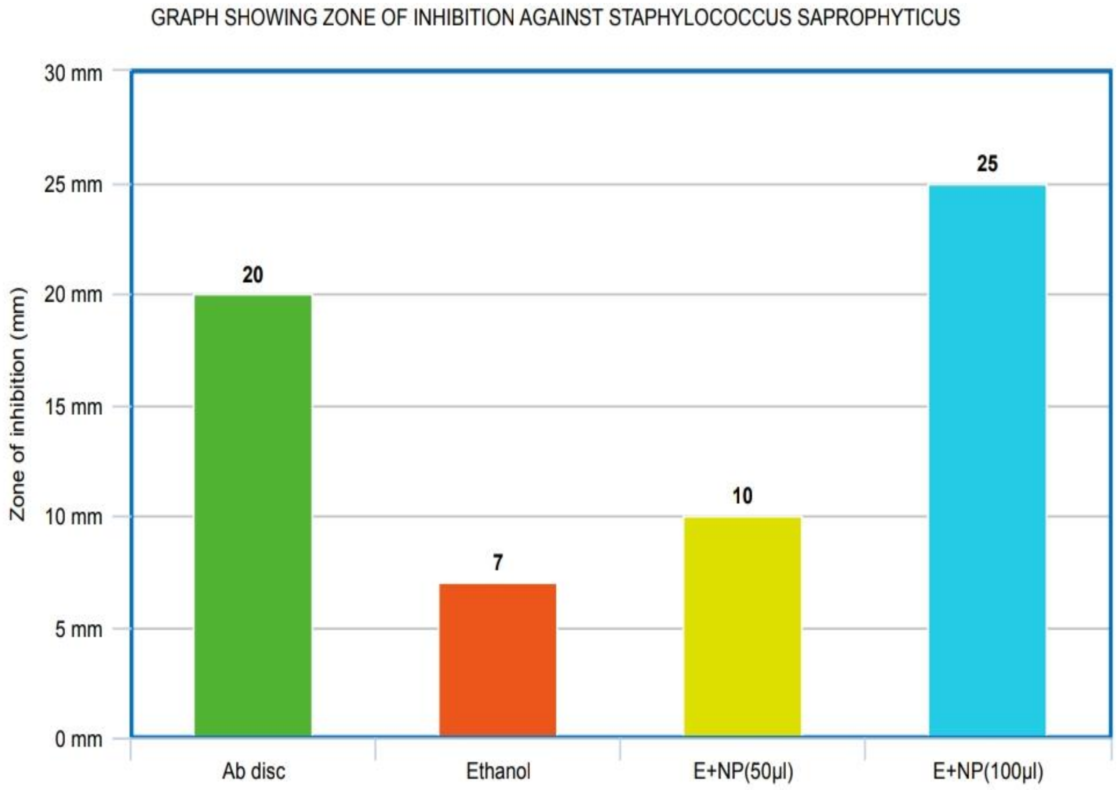
Graph 1: Bar Graph showing zone of inhibition against *E.coli*

Staphylococcus saprophyticus, commonly inhabiting the gastrointestinal and genital tracts of humans, is notorious for causing urinary tract infections (UTIs), especially in young sexually active women*.* *S. saprophyticus* is usually sensitive to antibiotics such as trimethoprim-sulfamethoxazole (TMP-SMX), nitrofurantoin, and fluoroquinolones, but antibiotic resistance can occur. However, like other bacteria, it can develop resistance to these antibiotics through various mechanisms, posing challenges in the treatment of infections caused by this bacterium. Antibacterial activity shown by the leaf extract of *Couroupita guianensis* against *Staphylococcus saprophyticus* is mentioned here (Table 2;figure xxxiii; bar graph 2).

Antibacterial disc	20mm
Ethanol	7mm
Ethanol +Nanoparticle(50µl)	10mm
Ethanol +Nanoparticle (100µl)	25mm

Table 2: Antibacterial effect shown against *Staphylococcus saprophyticus*



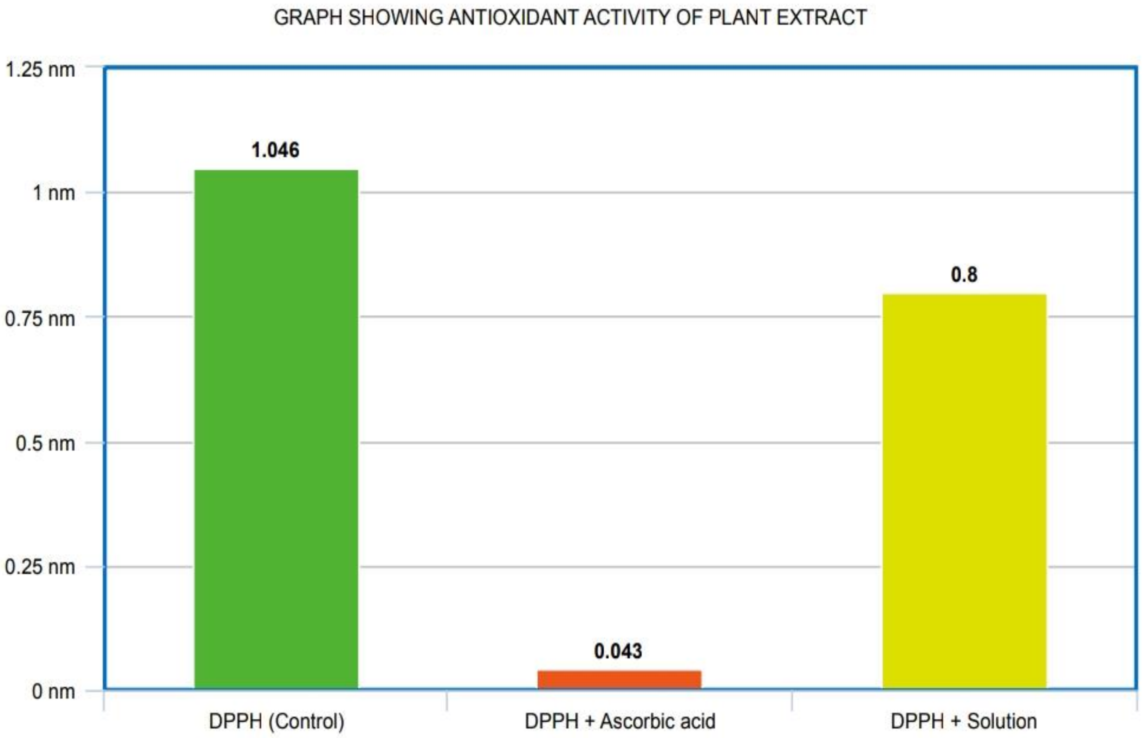


Graph 2: Bar Graph showing zone of inhibition against *Staphylococcus saprophyticus.*

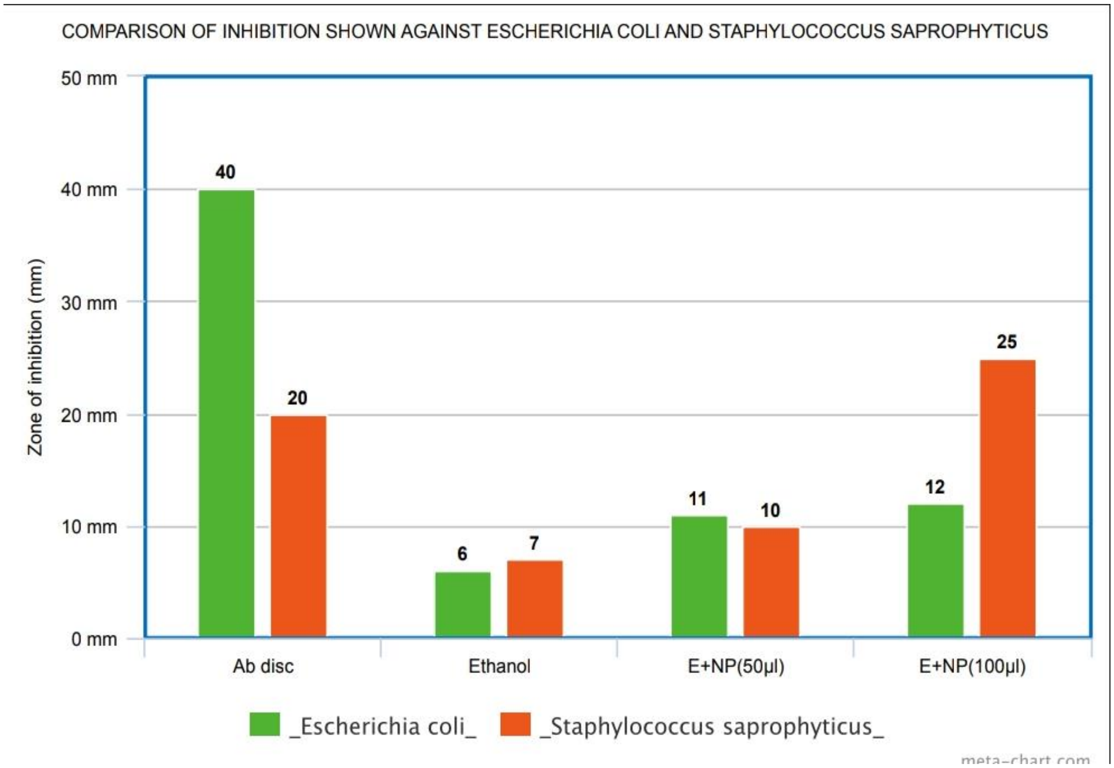
ZnO nanoparticles obtained from Couroupita guianensis demonstrate strong antioxidant properties (refer to Table 3, bar graph 3), holding promise for diverse applications in medicine and cosmetics. With their ability to effectively neutralize free radicals, these nanoparticles have the potential to alleviate oxidative stress-induced damage.

DPPH (Control)	1.046
DPPH + Ascorbic acid	0.043
DPPH + Solution	0.8

Table 3: Antioxidant activity of *Couroupita guianensis*



Graph 3 : Bar Graph exhibiting antioxidant property of *Couroupita guianensis.*



Graph 4 : Multiple bar diagram comparing the zone of inhibition against *E.coli* and *Staphylococcus saprophyticus*

In this study, we examined the synthesis of ZnO nanoparticles (NPs) using *Couroupita guianensis*, commonly known as the cannonball tree, is a fascinating area of study. These green synthesis methods are gaining attention due to their eco-friendliness and potential applications in various fields. In terms of antimicrobial effects, research indicates that ZnO NPs synthesized using *Couroupita guianensis* exhibit promising antibacterial and antifungal properties. These nanoparticles have shown effectiveness against a wide range of microorganisms, including bacteria like *Escherichia coli* and *Staphylococcus saprophyticus,*[31] as well as fungi such as *Candida albicans*. Moreover, the antioxidant properties of these synthesized ZnO NPs have also been investigated. Research indicates that they possess significant antioxidant activity, likely due to their capacity to scavenge free radicals and thwart oxidative damage[62]. This property holds significant potential for applications in the fields of medicine and cosmetics, where antioxidants are valued for their protective effects against various diseases and aging processes. Plant extract has a significant impact on the properties of nanoparticles as **(Mukunthan and Balaji, 2012).** The efficacy of these nanoparticles in inhibiting the activity of harmful microorganisms even at low levels has been demonstrated **(Applerot et al., 2009).** Studies have indicated that the use of plant extract for the bio-reduction[59,60] of relevant metal salts in nanoparticle synthesis can be achieved at room temperature. This method is considered to be less harsh and complex compared to traditional chemical bio-reduction processes.The beneficiaries of green synthesis of ZnONPs using Couroupita guianensis include:

(a) Environmentalists: Green synthesis reduces the use of harmful chemicals, minimizing environmental impact.

(b) Researchers: It offers a sustainable and cost-effective method for nanoparticle synthesis, expanding research possibilities.

(c) Industries: Access to eco-friendly synthesis methods can lead to the development of green products and technologies.

Green synthesis of zinc oxide nanoparticles (ZnONPs) using *Couroupita guianensis* holds significant promise in the field of nanotechnology due to its eco-friendly approach and potential applications. Several studies have investigated the synthesis process, properties, and applications of ZnONPs synthesized using *Couroupita guianensis* extract. The use of *Couroupita guianensis* extract as a reducing and capping agent for ZnONP synthesis eliminates the need for toxic chemicals, making the process environmentally benign **(Yadav, R., & Kala, S., 2020)**. ZnONPs synthesized using *Couroupita guianensis* extract exhibit potent antibacterial activity against various pathogens, making them promising candidates for biomedical applications such as wound healing and antimicrobial coatings **(Yadav, R., & Kala, S., 2020).** Studies have shown that ZnONPs synthesized using *Couroupita guianensis* extract demonstrate low cytotoxicity towards mammalian cells, suggesting their potential use in biomedical applications without adverse effects on human health **(Senthilkumar, S. R., et al., 2017).** ZnONPs synthesized using *Couroupita guianensis* extract exhibit excellent photocatalytic activity, making them suitable for environmental remediation applications such as wastewater treatment and air purification **(Vijayakumar, S., et al., 2017).**

The green synthesis of ZnONPs using Couroupita guianensis presents a sustainable and eco-friendly method for nanoparticle synthesis, with broad applications in biomedicine, environmental remediation, and beyond. This study's significance lies in its contribution to sustainable nanotechnology. By harnessing natural resources and eco-friendly processes, it advocates for environmentally conscious practices in nanomaterial synthesis, crucial for addressing environmental and health concerns linked to traditional synthesis methods.

CONCLUSION

Green synthesis methods like using Couroupita guianensis leaf extract for producing ZnO nanoparticles not only offer antimicrobial and antioxidant properties but also adhere to environmentally friendly practices. It's a great example of harnessing nature's resources for sustainable technology. Further research and testing are warranted to explore the full scope of its applications and evaluate its efficacy in real-world scenarios. Using natural plant extracts as reducing and stabilizing agents for synthesizing ZnONPs is indeed a sustainable and eco-friendly approach, reducing the environmental footprint compared to conventional chemical methods. It's a great example of leveraging nature's resources for greener technologies.*Couroupita guianensis*, commonly known as cannonball tree, is known to possess antioxidant properties due to its rich phytochemical composition. Studies suggest that the antioxidant activity of Couroupita guianensis may help combat oxidative stress-related diseases and contribute to overall health. Absolutely, continued research is crucial for uncovering the full spectrum of antioxidant effects and exploring the potential applications in both medicine and nutrition. It's an ongoing journey of discovery.

Our conclusion nicely summarizes the potential of the green synthesis of ZnO nanoparticles using Couroupita guianensis. It highlights the promising and eco-friendly nature of this approach and its potential applications across various fields. It's a great way to wrap up the discussion on the topic. The study demonstrated the effectiveness of the synthesis method in producing ZnO nanoparticles with desirable characteristics. The nanoparticles exhibited significant antimicrobial and antioxidant activities, highlighting their potential for use in biomedical and environmental applications. Further research can explore optimization strategies and elucidate the underlying mechanisms to enhance the synthesis process and maximize the nanoparticles' efficacy.

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