"Formulation of hand sanitizer using *Couroupita* guianensis and evaluation of its anti-microbial activity"

A RESEARCH PROJECT

Submitted by

SUYASH D. ZUNAKE HARSH K. BANGODI PRATIK S. MAGAR VISHAL B. GORE

UNDER THE GUIDANCE OF

Ms. SHWETA A. PISE

(Assistant Professor)

DEPARTMENT OF MICROBIOLOGY

VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2024-2025

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Ms. Shweta A. Pise

Research Project Guide

Examiner

Dr. T. C. Gaupale

Head of the Department

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Examiner Hand

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Head of the Department

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Research Project Guide

Dr. T. C. Gaupale

Head of the Department

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Place: Kolhapur

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Mr. Suyash D. Zunake

Mr. Harsh K. Bangodi

Mr. Pratik S. Magar

Mr. Vishal B. Gore

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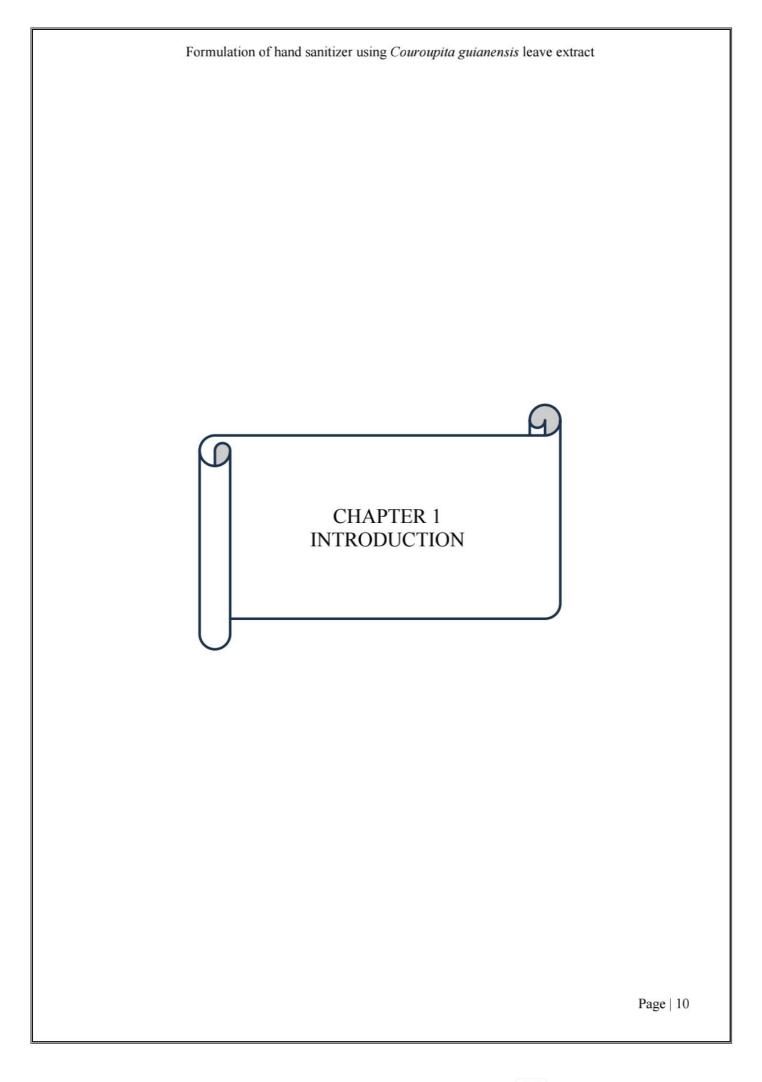
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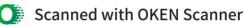
INTRODUCTION

Hands serve as a primary vector for the transmission of microorganisms and infectious agents. Consequently, hand hygiene is a fundamental practice in the prevention, control, and reduction of infections. Microbial flora present on the hands are broadly categorized into two types: resident and transient. Resident flora inhabit the deeper layers of the skin, particularly beneath the stratum corneum, and are also present on the skin surface.

In contrast, transient flora colonizes the superficial layers of the skin and are typically acquired through direct contact. These microorganisms are more susceptible to removal through routine hand hygiene practices. The presence and proliferation of transient flora are influenced by factors such as an individual's occupation, habits, and skin moisture. Healthcare workers are particularly at risk of hand colonization with pathogenic organisms, including *Staphylococcus aureus*, *Enterococcus spp.*, and yeasts, among others.

Routine handwashing may not effectively eliminate all harmful microorganisms; therefore, the use of alcohol-based hand sanitizers or antiseptics is recommended. During the COVID-19 pandemic, awareness of hand hygiene has significantly increased, and the use of hand sanitizers has become a widely adopted alternative to traditional handwashing with soap and water Medicinal plants hold immense value for humanity, spanning a broad spectrum of health, cultural, and economic benefit's. For millennia, they have been integral to traditional healing practices and remain a cornerstone of modern medicine.

Medicinal plants are rich sources of bioactive compounds that offer therapeutic effects against various health conditions. Phytochemicals found in these plants, such as alkaloids, flavonoids, tannins, and terpenoids, have shown properties like anti-inflammatory, antimicrobial, antioxidant, anticancer, and pain-relieving effects. Remedies derived from these plants are used to treat ailments ranging from common colds and digestive issues to chronic diseases like diabetes, hypertension, and cancer.



The use of medicinal plants offers a natural, often milder, and holistic approach to healthcare, focusing on treating the root cause of disease and improving overall well-being.

Medicinal plants have deep-rooted cultural and spiritual significance in many societies worldwide. Indigenous peoples have relied on their knowledge of medicinal plants for generations, using them in rituals, as tonics, and as remedies for both physical and mental illnesses. Traditional systems of medicine, such as Ayurveda, Traditional Chinese Medicine (TCM), and African herbal medicine, heavily incorporate medicinal plants in their therapeutic practices. These traditions are not only valuable in preserving cultural heritage but also provide important clues and empirical knowledge that inform modern medical research. (Kumar, C.S 2013)

Medicinal plants are a treasure trove for scientific research. The study of their chemical composition and mechanisms of action offers insights into disease processes, leads to the discovery of new therapeutic targets, and deepens our understanding of pharmacology and botany. Research on medicinal plants continues to yield new discoveries that inform drug development, alternative therapies, and integrative approaches to healthcare.

As interest in sustainable and natural lifestyles grows, medicinal plants are gaining popularity as alternatives to synthetic and processed health products. Many people prefer herbal remedies due to their perceived natural origin, lower toxicity, and environmental sustainability. This trend has led to a resurgence in the cultivation and use of medicinal plants, promoting sustainability in healthcare choices and the use of natural resources.

The increasing prevalence of antibiotic-resistant pathogens, harmful chemical pesticide residues, and skin-related health issues due to synthetic personal care products has necessitated a global shift towards eco-friendly, plant-based alternatives. In this context, *Couroupita guianensis*, commonly known as the Cannonball tree, has emerged as a promising medicinal plant owing to its rich phytochemical composition and wide range of bioactivities. Traditionally used in folk medicine, various parts of the *Couroupita guianensis* plant are known for their antimicrobial, antifungal, insecticidal, and anti-inflammatory properties.

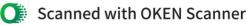
The present research project aimed to explore and scientifically validate the bioactive potential of Couroupita guianensis through a series of focused experiments. Alcoholic extraction of the plant was performed using a Soxhlet apparatus to ensure efficient recovery of phytochemicals. The

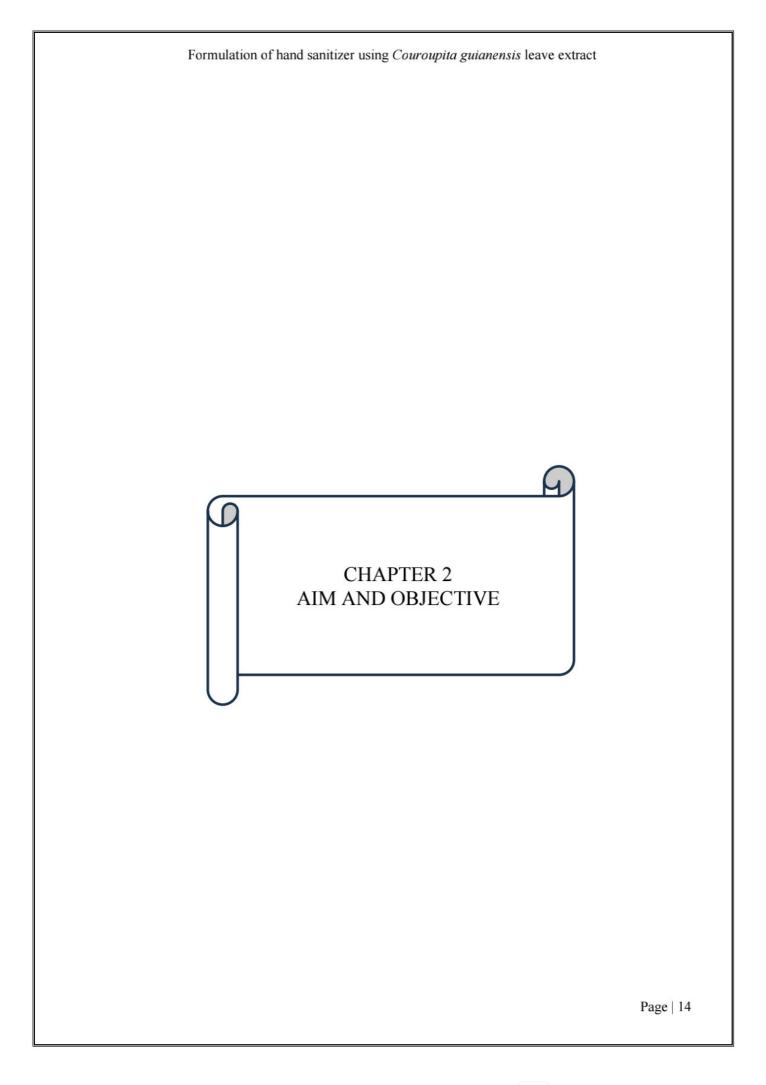
obtained extract was then subjected to various biological assays to assess its efficacy.

Antibacterial and antifungal screenings showed significant inhibitory activity against selected microbial strains, confirming the plant's potential as a natural antimicrobial agent.

In the final phase of the study, an herbal hand sanitizer was formulated using the alcoholic extract of Couroupita guianensis. Two variants Lavender and Lemon were developed, combining the antimicrobial strength of the extract with user-friendly aromatic formulations. The sanitizer demonstrated promising results in terms of germicidal efficacy, skin compatibility, and user acceptability.

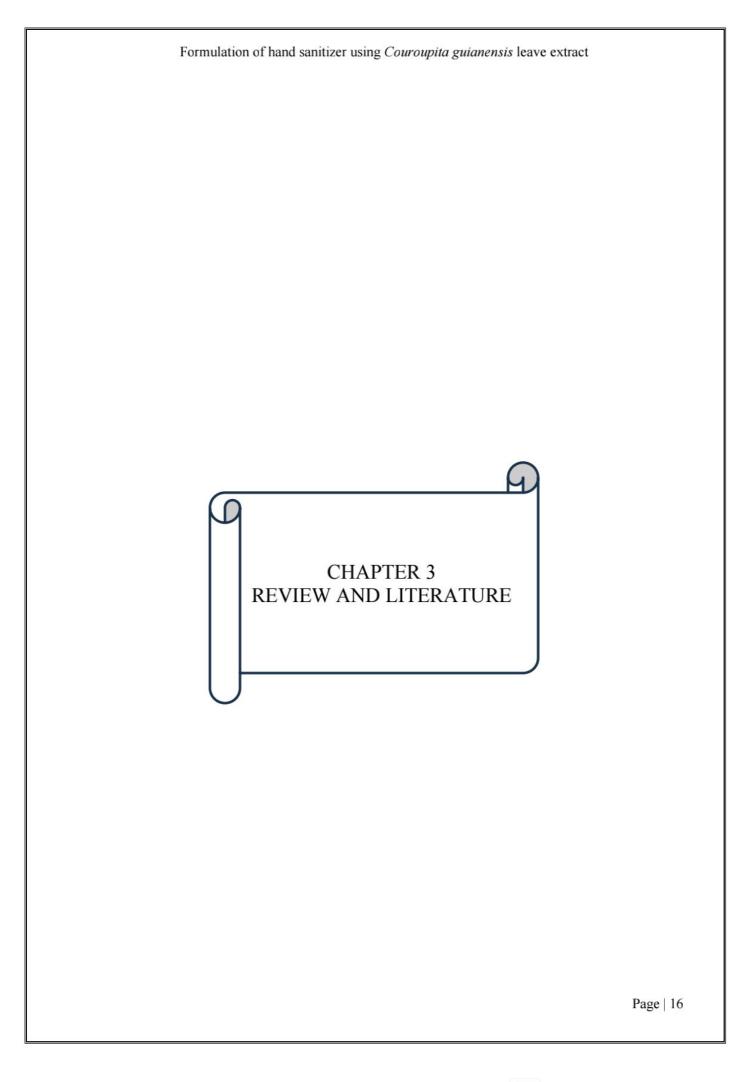
This research underscores the potential of Couroupita guianensis as a versatile plant for pharmaceutical, agricultural, and personal care applications. The outcomes provide a scientific foundation for further studies and commercialization of eco-friendly products derived from this medicinal plant.





AIM AND OBJECTIVES

- Aim- Formulation of hand sanitizer using Couroupita guianensis and evaluation of it's anti-microbial activity.
- Objectives-
- 1. To formulate hand sanitizer using Couroupita guianensis extract.
- 2. To evaluate anti-microbial efficiency of hand sanitizer.
- 3. To determine sensory analysis of hand sanitizer.



REVIEW AND LITERATURE

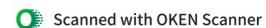
The widespread use of chemical disinfectants has been effective in reducing microbial load but is often associated with drawbacks such as skin irritation, unpleasant odors, and limited efficacy against bacterial endospores (Chowdary et al. 1908). Common chemical agents used include alcohols, halogens, aldehydes, and quaternary ammonium compounds, which work by mechanisms like protein coagulation and enzyme inhibition. However, due to their side effects, there has been a growing interest in herbal disinfectants, which are plant-based and environmentally friendly (1908).

Studies have shown that plants rich in saponins exhibit notable antimicrobial properties, providing an alternative to synthetic agents (1908). For example, *Costos igneus*, *Nepata cataria*, and *Tinospora cordifolia* have demonstrated high antimicrobial activity against pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli*, and *Klebsiella* species. According to *Nirmala Babu et al.*, the leaf extract of *Costos igneous* was highly active against *Bacillus subtilis* and *Candida parasitosis* at a low concentration of 2.5 µg/ml (1908). Similarly, Abalaka et al. and Ashfaq et al. identified the antimicrobial activity of neem leaves against major hospital pathogens (1908).

Agarwal et al. investigated Tinospora cordifolia extracts and observed a zone of inhibition of 19 mm against S. aureus, although in the current study, the inhibition was lower (9–12 mm), indicating variability in efficacy (1908). The emergence of multidrug-resistant (MDR) bacteria further underscores the importance of novel antimicrobial agents. Identifying such resistant strains in hospital environments is essential, and herbal disinfectants show potential in addressing MDR challenges with fewer side effects (1908).

Bachir, Raho G., and M. Benali. "Antibacterial Activity of the Essential Oils from the Leaves of *Eucalyptus globulus* against *Escherichia coli* and *Streptococcus aureus*." Asian Pacific Journal of Tropical Biomedicine, Sept. 2012.

Hand hygiene is a cornerstone in the prevention of infectious diseases, and alcohol-based hand



sanitizers have gained widespread acceptance due to their efficacy and ease of use.

The World Health Organization recommends alcohol-based formulations because they eliminate a broad spectrum of pathogens effectively. Recent developments have incorporated herbal components, enhancing the antimicrobial potential while minimizing the harsh effects of synthetic chemicals.

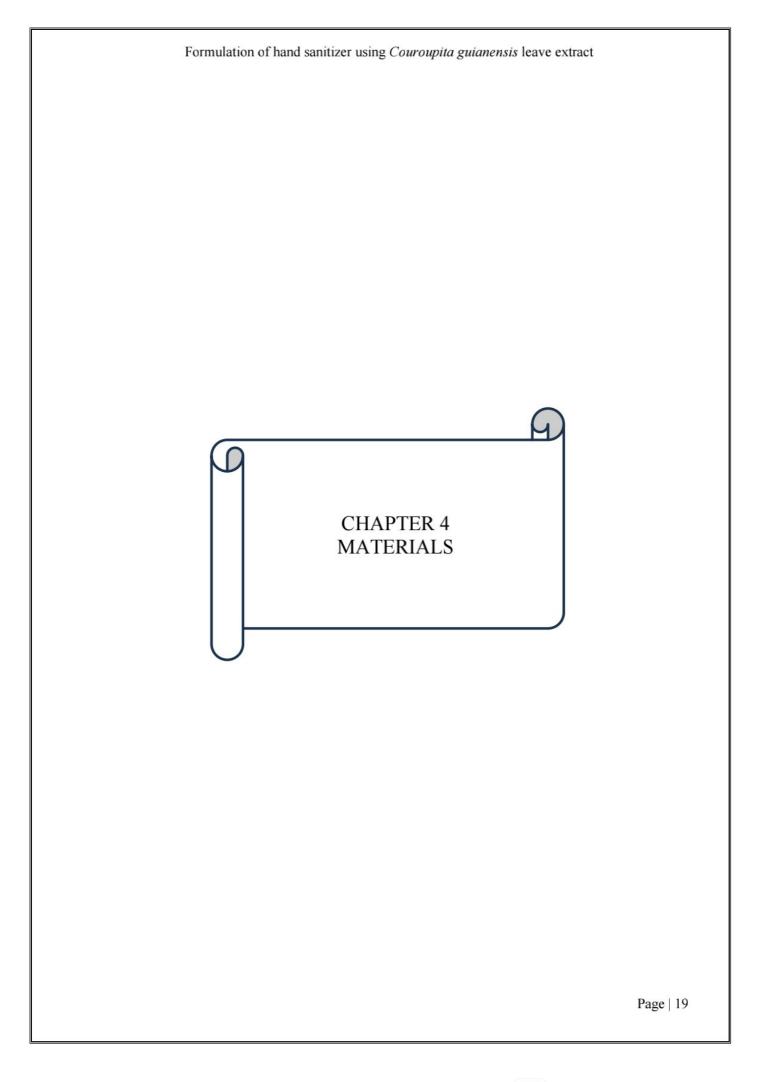
A key component in herbal sanitizers is eucalyptus oil, extracted from the leaves of *Eucalyptus globulus*. This essential oil is known for its antibacterial, antiviral, and deodorant properties. Bachir and Benali demonstrated the efficacy of eucalyptus oil against *Escherichia coli* and *Staphylococcus aureus*, making it an ideal agent for hand sanitizers (Bachir and Benali). Additionally, Brochot et al. explored essential oil blends and confirmed the strong antimicrobial and antiviral effects of eucalyptus-based preparations (Brochot et al.).

Hydrogen peroxide, another ingredient in the formulation, acts as an antiseptic and helps eliminate bacterial spores. Its inclusion enhances the microbial killing potential of the sanitizer (Medina-Cordoba et al.). Glycerol serves as a humectant, ensuring skin hydration and improving product acceptability, while orange peel oil adds aromatic value, enhancing the sensory experience without compromising functionality.

Previous studies emphasize the need for thorough evaluation of herbal hand sanitizers. Fatima Grace et al. and Gosh highlight the importance of assessing pH, clarity, odor, and skin compatibility to ensure both efficacy and safety (Grace et al.; Gosh). These considerations are critical for consumer use and regulatory approval.

Altogether, the literature supports the formulation of herbal hand sanitizers using components such as eucalyptus oil and hydrogen peroxide. These ingredients not only enhance antimicrobial activity but also maintain user comfort, making them practical solutions for hand hygiene in diverse settings.





MATERIAL

The present work was undertaken at Vivekananda college Kolhapur (An Empowered Autonomous

institute), Located in tarabai park, district Kolhapur.

1. Plant collection: Leaves of C. guianensis was collected from Vivekananda college garden,

Kolhapur, India.

2. Chemicals:

Ethanol (95%), Hydrogen peroxide were made available from the department of microbiology in

Vivekanand college and Glycerol(98%), Lemon oil, Lavender oil was bought from chemical shop

in Kolhapur. All this procedure was carried out in the department of microbiology of Vivekanand

college Kolhapur.

3. Media:

Nutrient agar

Composition: for 100ml media.

Peptone -0.5g

Beef extract -0.3g

Agar Agar - 1.5g

NaCl -0.5g

Distilled water-100ml

Formulation of hand sanitizer using Couroupita guianensis leave extract CHAPTER 5 **METHODOLOGY** Page | 21

METHODOLOGY

Preparation of Hand Sanitizer Formulations :

Initially, 3% hydrogen peroxide was added to a beaker containing 95% ethanol and placed on a magnetic stirrer to ensure uniform mixing. Glycerol (98%) was then added gradually to the mixture under continuous stirring until a homogenous solution was obtained.

The active ingredient, *Couroupita guianensis* powder, was incorporated into the formulation in two different concentrations:

- Formulation A: 10 g of C. guianensis powder was added to 140 ml of ethanol.
- **Formulation B:** 10 g of *C. guianensis* powder was added to 180 ml of ethanol.

Each formulation was prepared in two variations by incorporating either Lavender oil or Lemon oil (lab grade) as fragrance agents.

After the addition of essential oils, the volume of each mixture was adjusted to 50 ml using sterile distilled water to obtain the final formulation. Thus, a total of four hand sanitizer variants were prepared:

Formulation A with lavender oil

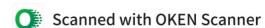
Formulation A with lemon oil

Formulation B with lavender oil

Formulation B with lemon oil

All samples were stored in sterile containers for further analysis and evaluation.

The efficiency of hand sanitizer is checked against different micro-organisms.



[Formulations F1 and F2 were formulated by using extract prepared from 10 gm of plant powder in 140 ml of ethanol while formulation F3 and F4 were formulated by using extract prepared from 10 gm of plant powder in 180 ml of ethanol.]

Table 1: Formulation composition of herbal hand sanitizer.

Formulations	F1 (ml)	F2 (ml)	F3 (ml)	F4 (ml)
Extract	0.5	O.5	0.5	0.5
Ethanol (95%)	35	35	35	35
H2O2 (3%)	1.5	1.5	1.5	1.5
Glycerol (98%)	1	1	1	1
D/w	11	11	11	11
Essential oil (1ml)	Lavender	Lemon	Lavender	Lemon

Table 2: Role of ingredient used in formulation

Ingredients	Strength	Category
_		
Extract	10 gm powder in 140 ml and	Anti-bacterial, Anti-fungal
	180 ml ethanol	properties
Ethanol	95%	Surface disinfectant
H2O2	3%	Antiseptic, antibacterial and
		anti-spores
Glycerol	98%	Skin care and to increase
		acceptability of the product.
Essential oil	93.5%	Fragrance
Distilled water	100%	Vehicle

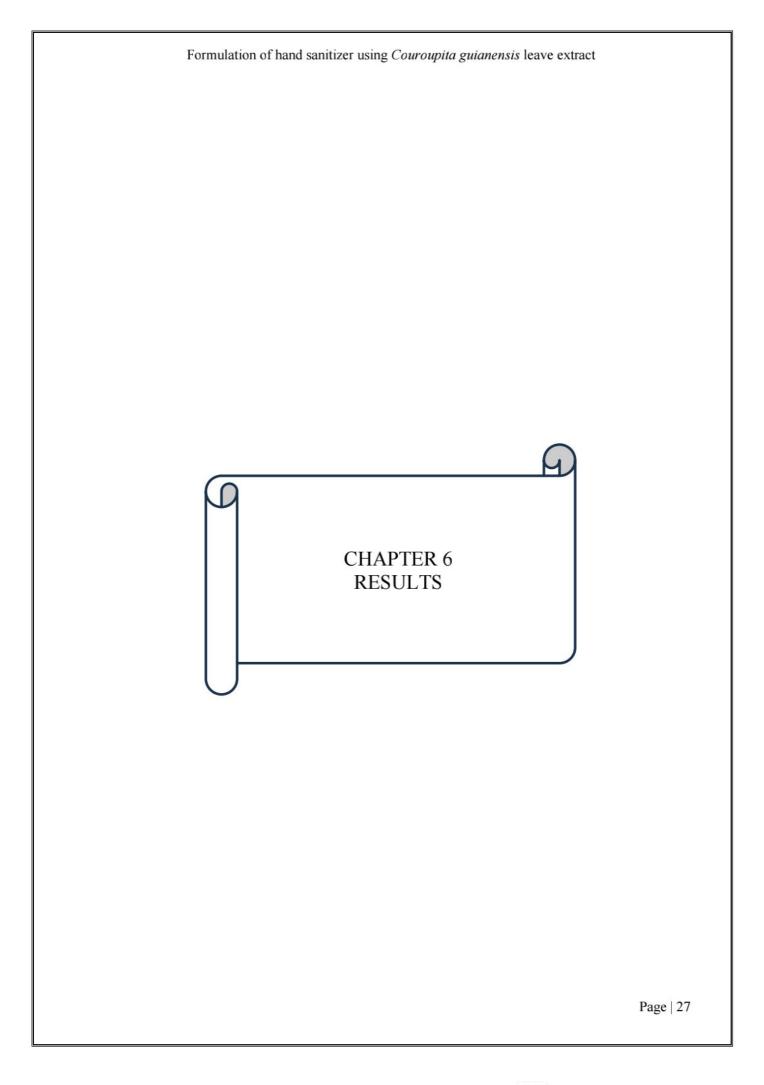
Evaluation test for herbal hand sanitizer:

1. Appearance:

- I. Odor: It was determined manually.
- ii. Color: It was determined visually.
- 2. Determination of pH: The pH of herbal hand sanitizer was determined by using Digital pH meter.
- **3.** Clarity: Clarity test was determined to evaluate presence of particulate matter visually.
- **4. Skin Irritation Test:** Skin Irritancy of hand sanitizer was evaluated by taking small amount of formulation on palm. Checked for local irritation or any inflammatory reactions (if present or not).
- 5. IN-VITRO Anti-microbial activity by Nutrient Agar well Plate method: The screening of Anti-microbial efficiency of Formulation F1, F2, F3, F4 were performed on organism using agar well method as per standard procedure. 20 sterile petri plates were taken for evaluating activity against 5 microorganisms. Sterile Nutrient agar prepared and autoclaved at 121°C to 123°C for 15 min at 15lb pressure. Plates were filled with Nutrient Agar solution and kept for solidification. After solidification plates were inoculated with microorganism. Wells were made into the agar plates for pouring sample in the well. Now the plates were incubated at 37°C and after 24 hrs. Of incubation, plates were observed for zone of Inhibition. Responses of organism to formulations were measured.

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6. Media: Nutrient Agar Medium. Pathogen selected for anti-microbial property is listed below: Bacterial Strains used: 1. S. aureus 2. K. pneumonia 3. E. coli 4. Salmonella spp. 5. Bacillus spp.	Formulation of hand sanitizer using Couroupita guianensis leave extract	
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 S. aureus K. pneumonia E. coli Salmonella spp. 	Pathogen selected for anti-microbial property is listed below:	
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3. E. coli 4. Salmonella spp.		
4. Salmonella spp.		
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RESULTS

Table 3: Results of evaluation parameters

Evaluation parameter	Observation
Odour	Pleasant
Color	Greenish
pH	6.5 +/- 0.1
Clarity testing	Clear solution
Skin irritation test	No irritation observed

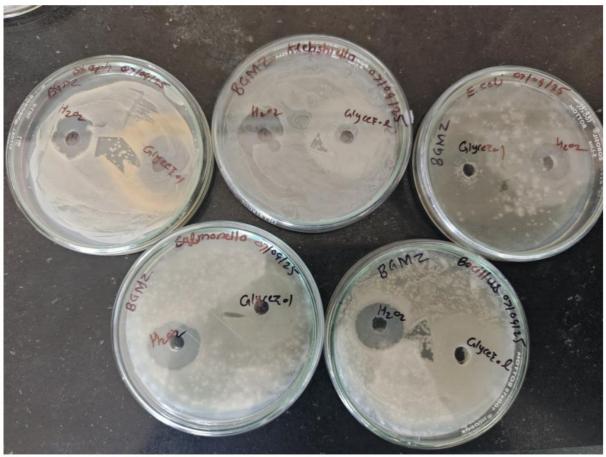
Table 3 represents the results of key evaluation parameters for the formulated product. The assessments include organoleptic properties, pH, clarity, and safety through a skin irritation test, providing insights into the product's quality, stability, and compatibility with skin

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Table 4: Result of activity of microorganisms against ingredients

Microorganism	Zone of inhibition of H2O2 (3%) in mm.	Zone of inhibition of Glycerol (98%) in mm.
S. aureus	22	27
K. pneumonia	23	11
E. coli	20	-
Salmonella spp.	25	-
Bacillus spp.	26	-

The results from table no. 4 indicate that both hydrogen peroxide (3%) and glycerol (98%) exhibit antimicrobial activity, with varying zones of inhibition against different microorganisms. Glycerol showed particularly strong activity against *S. aureus* and *K. pneumonia*, suggesting its potential as an effective antimicrobial agent in the formulation along with H2O2.

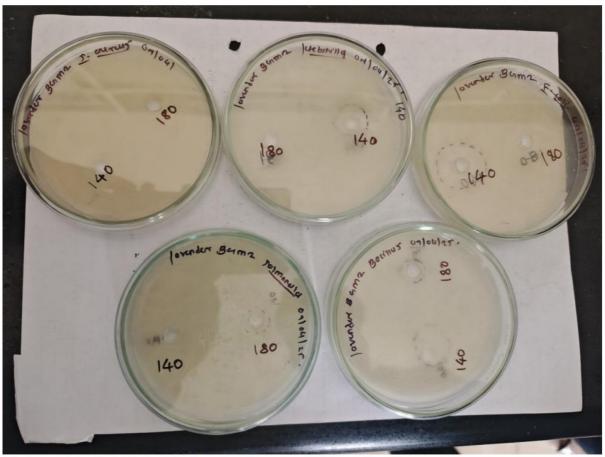


Photograph no.1 Activity of microorganisms against ingredients.

Table 5: Results of activity of Microorganisms against F1 and F3 formulation (Lavender)

Microorganism	Zone of inhibition of F1 in mm	Zone of inhibition of F3 in mm
S. aureus	6	0
K. pneumonia	19	0
E. coli	18	0
Salmonella spp.	0	14
Bacillus spp.	21	12

The results from table no. 5 indicate that F1 formulation showed very strong antimicrobial activity, particularly against *K. pneumonia*, *E. coli*, and *Bacillus spp.*, while F3 (Lavender-based) exhibited no significant inhibition, indicating limited or no antimicrobial effect.

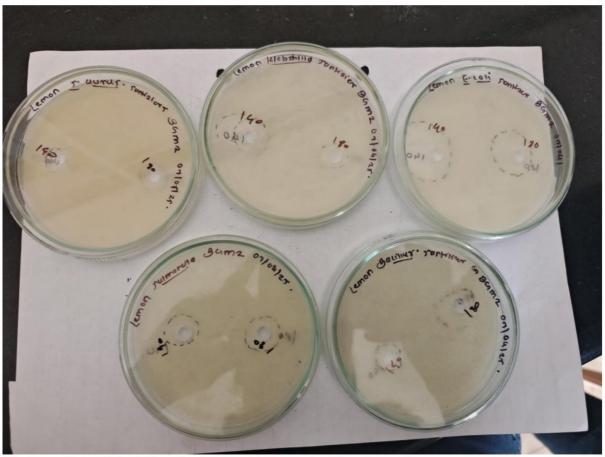


Photograph no.2 Activity of microorganisms against lavender oil.

Table 6: Results of activity of Microorganisms against F2 and F4 formulation (Lemon)

Microorganism	Zone of inhibition of F2 in mm	Zone of inhibition of F4 in mm
S. aureus	13	16
K. pneumonia	20	16
E. coli	28	26
Salmonella spp.	16	17
Bacillus spp.	14	16

The results from table no.6 indicates that, Both F2 and F4 (Lemon-based) formulations demonstrated strong antimicrobial activity, with F2 and F4 showing slightly higher zones of inhibition, particularly against *E.coli* indicating enhanced effectiveness of the formulation.

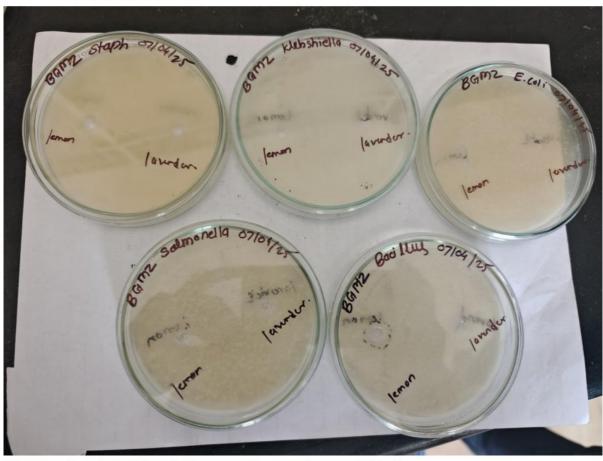


Photography no.3 Activity of microorganisms against lemon sanitizer.

Table 7: Results of activity of Microorganisms against essential oils

Microorganism	Zone of inhibition of Lemon oil in mm	Zone of inhibition of Lavender oil in mm
S. aureus	-	-
K. pneumonia	-	-
E. coli	-	-
Salmonella spp.	-	-
Bacillus spp.	19	-

The results from table no. 7 indicate that, there is no zone of inhibition against both lemon oil and lavender oil. It shows that the role of essential oils is only to give aroma.



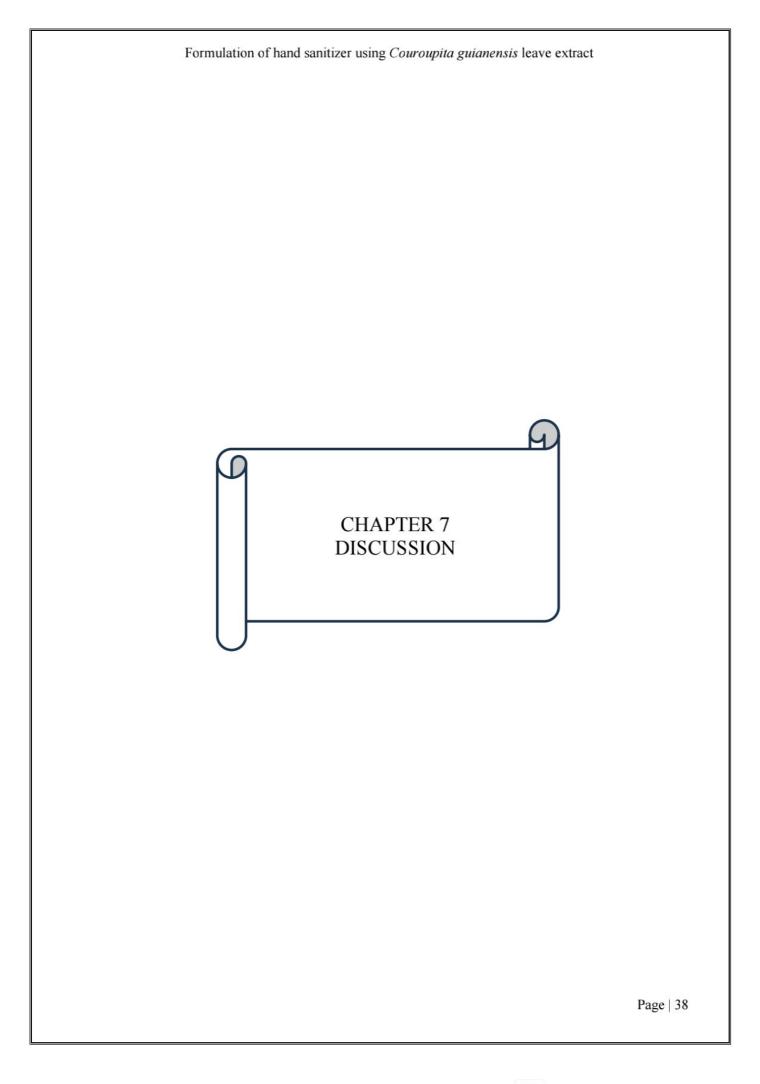
Photograph no.4 Activity of microorganisms against essential oils.

Table no. 8 Sensory testing of herbal hand sanitizer.

Sensory testing of herbal hand sanitizer was performed by 10 untrained persons.

Attribute	Description	Score Range	Average Score of 10 people
Appearance / Clarity	Visual clarity and Color consistency	1(poor)-5(excellent)	3
Odour/ Aroma	Herbal scent strength and pleasantness	1(unpleasant)- 5(very pleasant)	5
Texture / Feel on Hands	Sensation during and after application	1(sticky or irritating)- 5(smooth or clean)	4
Absorption	Speed and completeness of absorption.	1(slow or poor)- 5(quick or complete)	4
Residue	Presence of any sticky or oily residue.	1(high residue)-5(no residue)	3
Overall Acceptability	General satisfaction with the product.	1(not acceptable)- 5(highly acceptable)	4

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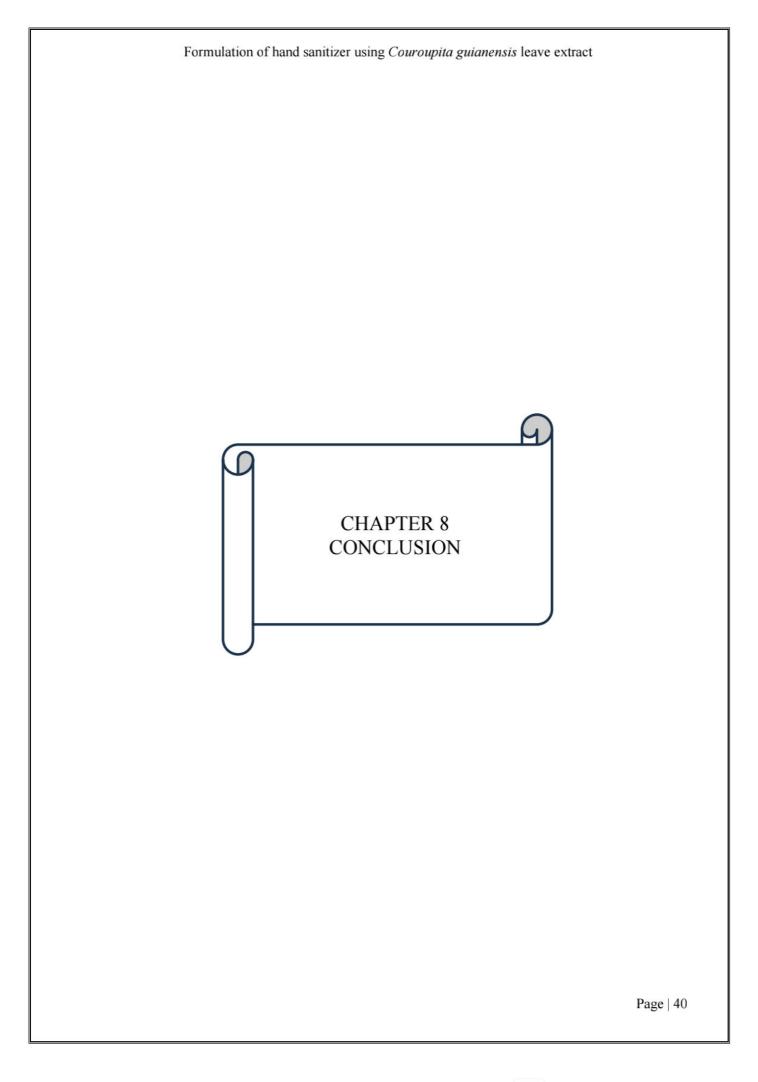


DISCUSSION

The present research focused on the medicinal potential of *Couroupita guianensis* (commonly known as the Cannonball tree), a plant known for its rich phytochemical composition and traditional therapeutic uses. Initially, a comprehensive study of the plant was conducted to understand its morphological characteristics, traditional applications, and the presence of bioactive compounds. Alcoholic extraction of the plant material was carried out using a Soxhlet apparatus, which efficiently isolated the active constituents believed to be responsible for the plant's medicinal properties.

The resulting extract was subjected to antimicrobial analysis, where it demonstrated significant antibacterial and antifungal activity against selected strains. These findings highlight the potential of *C. guianensis* as a natural antimicrobial agent. Further testing revealed notable antilarval activity, suggesting possible applications in mosquito control and public health interventions.

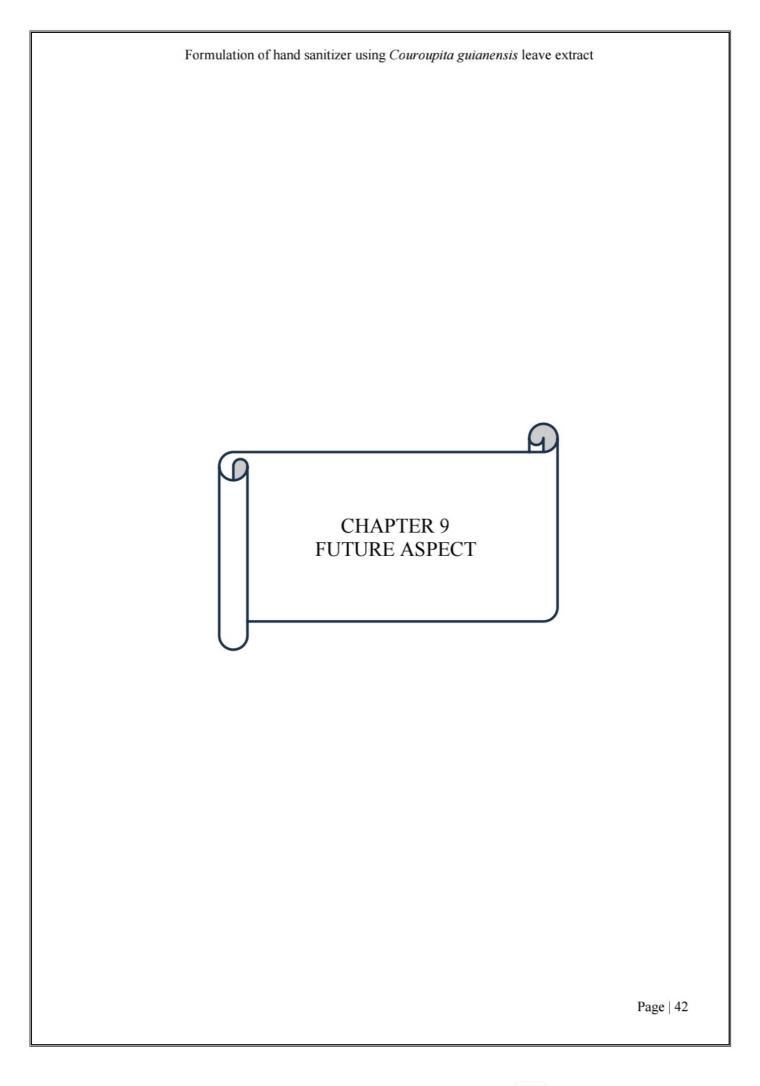
To explore practical applications, a herbal hand sanitizer was formulated using the alcoholic extract. Two variants were developed, incorporating lavender and lemon essential oils for fragrance and additional antimicrobial benefits. The final product aims to offer a natural and effective alternative to chemical-based sanitizers, aligning with current demands for herbal and eco-friendly healthcare products. This research underlines the value of *Couroupita guianensis* in developing multifunctional herbal formulations for personal and public health.



CONCLUSION

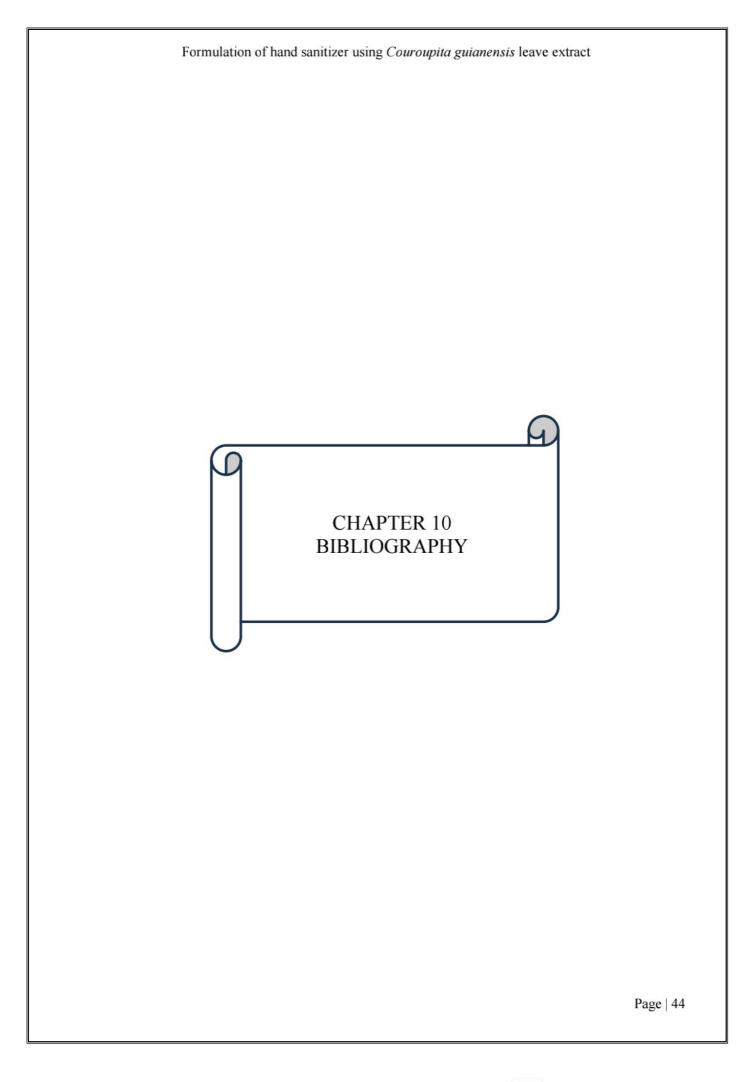
The present study on Couroupita guianensis has demonstrated its significant potential as a source of bioactive compounds with multiple applications. The initial phase involved the systematic study and identification of the plant species, followed by the preparation of an alcohol-based extract using Soxhlet apparatus, which efficiently isolated phytoconstituents with potential biological activity.

A novel value-added product, herbal hand sanitizer, was successfully formulated from the extract in two aromatic variants, Lavender and Lemon. This product offers a natural, effective, and eco-friendly alternative to synthetic sanitizers, aligning with consumer demand for herbal and chemical-free solutions.



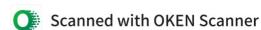
FUTURE ASPECT

The research opens avenues for large-scale production and commercialization of bio-based products derived from Couroupita guianensis. Further studies involving phytochemical profiling, toxicity analysis, and clinical validation are essential to ensure safety and efficacy. The plant extract can also be explored in integrated pest management (IPM) programs and as a natural preservative or disinfectant. With increasing global interest in green technologies, this research lays a strong foundation for sustainable, plant-based innovations in agriculture, public health, and personal care industries.

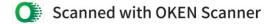


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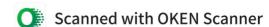


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