"SCREENING OF ANTIMICROBIAL ACTIVITY, PHYTOCHEMICAL ANALYSIS AND

ANTIHELMINTHIC ACTIVITY OF COUROUPITA

GUIANENSIS"

A RESEARCH PROJECT

Submitted by

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VIVEKANAND COLLEGE, KOLHAPUR

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YEAR 2024-2025

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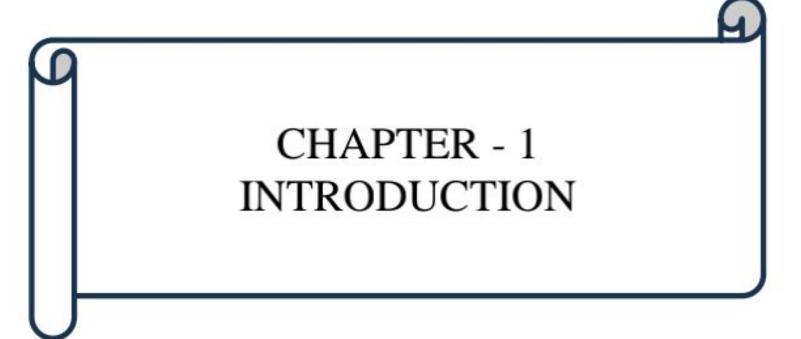
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INTRODUCTION

Medicinal plants hold immense value for humanity, spanning a broad spectrum of health, cultural, and economic benefits. 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)

A significant number of modern pharmaceutical drugs are derived from or inspired by compounds found in medicinal plants. For example, aspirin originated from salicylic acid found in willow bark, and the anti-cancer drug paclitaxel (Taxol) was developed from the Pacific yew tree. Approximately 25% of modern medicines are plant-based, and many more synthetic drugs are modeled after plant compounds (Gousia, S.K.2013). The exploration of medicinal plants provides a continuous source of new drug leads, driving innovation and expansion in pharmacology.

For many people, particularly in developing countries, medicinal plants are a vital source of accessible and affordable healthcare. Synthetic drugs can be expensive and sometimes unavailable, making natural remedies derived from local flora more practical for treating illnesses. This reliance on medicinal plants for primary healthcare underscores their critical role in global health systems, offering cost-effective solutions with minimal side effects when used properly.

The trade of medicinal plants represents a significant sector in global and local economies. Many rural communities rely on harvesting and selling medicinal plants as their primary livelihood. The global demand for herbal medicines, supplements, and natural products has surged in recent years, boosting the economic value of medicinal plants.(R.singh.2015) The cultivation, processing, and sale of these plants can help generate income, support rural economies, and contribute to national growth.

The value of medicinal plants extends to their role in conservation and biodiversity. Many medicinal plants are integral components of their ecosystems, contributing to ecological balance and maintaining biodiversity. Efforts to cultivate and sustainably harvest medicinal plants can promote conservation initiatives, protect endangered species, and encourage the preservation of plant habitats(.R Singh2015.) This ecological value ensures that medicinal plants remain available for future generations while maintaining the health of our planet's ecosystems.

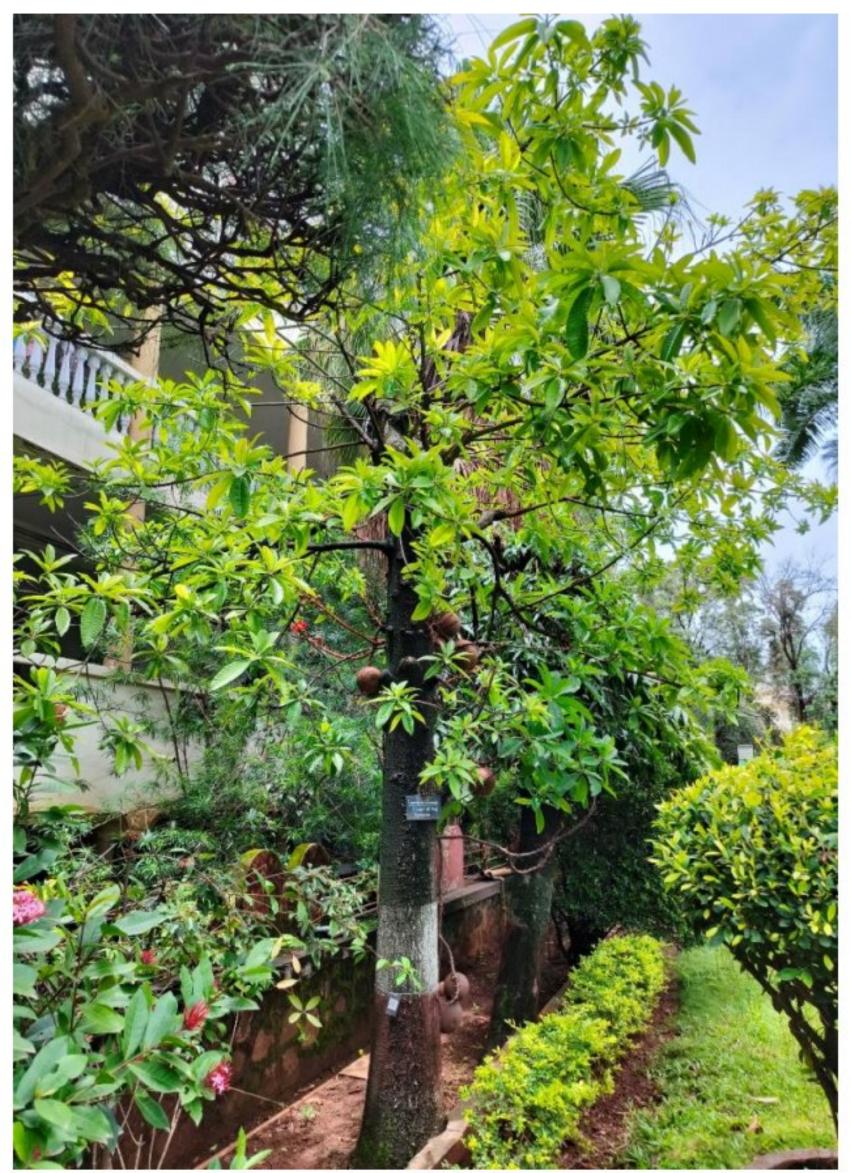
Medicinal plants are a treasure trove for scientific research. The study of their chemical composition and mechanisms of action offers insights into disease processes, leads to the discovery of new therapeutic targets, and deepens our understanding of pharmacology and botany. Research on medicinal plants 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

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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.

In summary, the value of medicinal plants is immense and multifaceted. They serve as the foundation of traditional medicine, fuel discoveries in modern medicine, provide accessible healthcare, support livelihoods, and contribute to biodiversity and environmental conservation. Their continued study and preservation are vital for the health and well-being of humanity and the planet.



Photograph 1CANNON BALL TREE

The cannonball tree, scientifically known as *Couroupita guianensis*, is a fascinating and unique flowering species native to tropical regions of South America, particularly the rainforests of the Amazon Basin. Belonging to the family Lecythidaceae, this remarkable tree has garnered attention and admiration across the world, not only for its striking appearance and distinctive fruits but also for its deep cultural, medicinal, and ecological significance (Sheba.l.A2019). Named for its large, spherical fruits that resemble rusty cannonballs, this tree thrives in humid climates and has been widely cultivated in various tropical and subtropical areas, including parts of India, Sri Lanka, Southeast Asia, and the Caribbean.

The cannonball tree can reach impressive heights of up to 25 meters (approximately 82 feet) and

boasts a thick, straight trunk often covered in blossoms and fruits. Unlike many other trees, its flowers grow directly from the trunk, a trait known as "cauliflory." This unique adaptation allows pollinators such as bees and bats to access the flowers with ease. The flowers themselves are strikingly beautiful, characterized by large, waxy petals ranging in shades from deep pink to vibrant red, with a distinctive, almost musky fragrance that attracts pollinators. Each flower has a complex structure with a central hood-like formation, which plays a crucial role in reproduction and the attraction of insects.

One of the most eye-catching aspects of the cannonball tree is its massive, woody fruits that resemble cannonballs, measuring up to 25 centimeters (10 inches) in diameter.

When mature, these fruits contain numerous seeds embedded in a pulp with a strong odor.

Although inedible to humans due to the pungent smell and hard shell, the fruit serves as an important source of food for various animals, including rodents and other forest-dwelling creatures that aid in seed dispersal. The fruits take several months to mature and eventually fall to the ground with a loud thud, often splitting open upon impact.

The cannonball tree holds cultural and spiritual importance in many parts of the world, particularly in India and Sri Lanka, where it is often planted near temples and revered for its sacred associations. In Hinduism, the tree is linked to Shiva, and its flowers are sometimes used in religious offerings. Beyond its spiritual significance, the cannonball tree has been used in traditional medicine for centuries. Indigenous communities have long utilized various parts of the tree for their medicinal properties; for example, extracts from the flowers, leaves, and bark are believed to have anti-inflammatory, antimicrobial, and analgesic properties. (These natural remedies are employed to treat ailments ranging from skin conditions to digestive disorders) (Rajeshwari V.D 2014).

In addition to its ornamental and medicinal uses, the cannonball tree plays an important ecological role within its natural habitat. By providing a reliable food source for pollinators and wildlife, it helps maintain the balance of tropical forest ecosystems. Furthermore, its unique flowering and fruiting behavior offers a fascinating case study for botanists and ecologists

interested in plant-pollinator interactions and cauliflorous adaptations.

In recent years, the cannonball tree has become a popular feature in botanical gardens and parks around the world, where it continues to amaze visitors with its unusual characteristics and dramatic appearance. Its striking combination of large, fragrant flowers, impressive fruits, and rich historical background make it a subject of great interest to plant enthusiasts, researchers, and cultural historians alike.

Whether admired for its beauty, studied for its unique biological features, or valued for its traditional uses, the cannon ball stands as a testament to the incredible diversity and complexity of the plant kingdom.

SCIENTIFIC CLASSIFICATION OF COUROPITA GUIANENSIS

Kingdom: Plantae

Division: Angiosperms

Class: Eudicots

Order: Malpighiales

Family: Lecythidaceae

Genus: Couroupita

Species: C. guianensis

CHAPTER -2 AIM AND OBJECTIVE

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AIM AND OBJECTIVE

Aim- To study the antimicrobial, phytochemical and anthelminthic activity of Couroupita
guianensis.

Objectives –

- 1. Determination of the antimicrobial activity of Couroupita guianensis.
- 2. Phytochemical analysis of Couroupita guianensis.
- 3. Anthelmintic activity of Couroupita guianensis.

CHAPTER – 3 REVIEW AND LITERATURE

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REVIEW AND LITERATURE

For thousands of years, plants have formed basis of medicinal treatments and remedies. Use of natural products for therapeutic purposes is as old as human utilization. People have used plants as food and powder for healing, to prevent disease. The increase in microbial antibiotic resistance on the global scale emphasizes the development of new antibiotics. As a result, the use of antioxidants is as interest today. Natural antioxidants known to have wide range of biological effect. The important plant is use in traditional medicine is Couroupita guianensis, contain oil, keto, steroids, glycosides. Couroupita, indirubin, isatins and phenolic substance. Plant leaves were collected, dried and powered and used for further phytochemical analysis. Extraction was done by using ethanol, chloroform and aqueous solution. Using extract antimicrobial activity. The methanolic extraxtion shows maximum antimicrobial activity i.e.. Zone inhibition. Thus, activity reveals that active component have been extracted in methanol. So the present study formulated to average antimicrobial aspect. (Kavita. kamalakannan, 2011).

Plant have been central to the development of modern medicine with nearly all plants parts such as leaves flowers roots and seeds, demonstring diverse medicinal properties. The increasing reliance an natural products has spurred active screening of plant extracts for novel drug discoveries with application in pharmaceutical industries cosmetics agriculture and food industries. *C. guainensis* commonly known as the cannonball tree or Nagalingam push am is native to south India and Malaysia and has rich history in traditional medicine. The tree produces distinctive fragment flowers and large woody fruits and its part are known to contain bioactive compounds like phenolics, glycosides etc. these compounds exhibit a wide range of medicinal properties, including antibacterial, antifungal, antioxidant and anti-inflammatory activities.

Ethanolic extracts are highly effective against earth worms than aqueous extract, which is useful in treatment against helminthic infections. The extensive literature survey revealed that Couroupita guianensis is an important medicine as plant with diverse. Pharmacological uses. They possess antibiotic, antiseptic and analgesic qualities.(S.k.gausia 2013)

C. guainensis or the cannonball tree, is native to south India, called for its medicinal and cultural significance. Revered in Hindu and Buddhist traditional all parts of the tree possess therapeutic properties different parts of plant such as leaf, flowers, bark use as a source of medicinal agent.

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Leaf extraction is carried out in different solvents like, chloroform, ethanol and water. Methanolic extracts show antimicrobial, antifungal and anticancer effects, while antioxidants rich young flowers and fruits aid wound healing. Ethanol extracts prevents gastric lesions, and the plant exhibits anthelmintic activity similar to drug albendazole and anti inflammatory properties rival paracetamol also its leaf extracts show antidiabetic potential .The plant also boosts immunity making it a promising candidate for drug development with minimal side effect . in short in future it seems be attractive niche for the scientist and proves for mankind.(vivbek.p.Chavda2015)

CHAPTER- 4 MATERIAL AND METHOD

MATERIALS

The present work was undertaken at Vivekananda college Kolhapur (Empowered Autonomous), Located in tarabai park, district Kolhapur.

- 1. **Plant collection**: Leaves of *C. guianensis* was collected from Vivekananda college garden, Kolhapur, India.
- Chemicals: Ethanol, ferric chloride, potassium iodide, NAOH, concentrated HCL, lead
 acetic acid, magnesium ribbon, ammonium sulphate, Copper sulphate, biuret reagent,
 starch reagent and all of this chemicals and reagent was collected from department of
 microbiology and chemistry department of Vivekanand college Kolhapur.

3. Earthworms:

Species: Eisenia fetida.

This species was collected from Sachin Jadhav, (Ruikar colony, near HP gas station, Kolhapur).

- 4. Media:
- 1. Nutrient agar
- 2. MacConkey's agar
- 3. Potato dextrose agar

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4. Muller Hinton agar

Beef Extract – 3 g

Acid Hydrolysate of Casein - 17.5 g

Starch - 1.5 g

Agar – 15 g

Sodium Chloride (NaCl) – 5 g

Distilled Water - 1 L

METHODS

1. Testing for antimicrobial activity.

The antimicrobial activity of *Couroupita guianensis* was investigated using the agar well diffusion method. In this study, the leaf extract of *Couroupita guianensis* was evaluated for its inhibitory effects on both gram positive and gram-negative organisms and fungal spp.

The methodology involved spreading a 24hrs old fresh culture (suspension) of the organism onto sterile nutrient agar and muller Hinton agar plates, followed by creations of wells using a sterile cork borer(s.manimeglai.2014). A 0.1 ml sample of the leaf extract was then added to each well. The plates were refrigerated for 10 min. for diffusion of the sample, often which were incubated at 37 degrees Celsius for 24 hours.

After incubation, plates were examined for zones of growth inhibition, and the diameter of this zone was measured. The result is presented in table no. 2, while the zone of inhibition showed in fig......

2. Test for phytochemical analysis.

Ethanolic extraction was used for phytochemical screening.

For preparation of ethanolic extraction 10gm of leaf powder was soaked into 100ml of (95%) ethanol. And kept on rotary shaking for 6-8 hours.

Phytochemical investigation of c. guianensis revealed in the presence of carbohydrates, terpenoids, glucosides, alkaloids, steroids, tannins, phenols, proteins.

Test for Alkaloids

 Mayer's test: Test solution treated with Mayer's reagent (Potassium mercuric iodide) gives cream colored precipitate.

Test for protein

- Biuret test: Test solutions treated with 40% sodium hydroxide and dilute copper sulphate solutions give blue color.
- Test for tannins
- 1. Gelatine test: plant extract dissolved in 5ml distilled water.(gives blue color).

Test for Phenols

I. Ferric Chloride Test: A small amount of the ethanolic extract was taken with I mL of water in a test tube and I to 2 drops of Iron Ill chloride (FeC13) was added. A blue, green, red or purple color is a positive test.

Test for Terpenoids

1. Salkowski test: When few drops of concentrated sulphuric acid is added to the test solution, shaken and allowed to stand, lower layer turns red indicating the presence of sterols.

Test for Carbohydrates

1. reagent (Saturated picric acid solution) gives yellow precipitate.

Test for Steroids

.

 Salkowski's test: The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown color.

2. Evaluation of anthelminthic activity.

"Anthelmintic activity of *Couroupita guianensis* leaf extract was evaluated against Indian adult earthworm.

In this study, the anthelmintic activity of c. guianensis leaf extract was evaluated against Indian earthworms. The experiment was conducted using various extract solutions, including ethanolic aqueous, and standard drug albendazole syrup.

Methodology:

- All extract solutions were prepared prior to the experiment.
- 10ml of each extract solution and std. drug albendazole syrup were poured into separate petri plates.
- Earthworms were wasted on a normal saline solution. And added to each plate (5 earthworms to each plate).
- Observations were made for time taken to paralysis and death(sacrifice) of the earthworms.
- The volume of each extract solution was adjusted to 10ml.
- Observed that in ethanol extraction, paralysis occurred within 25 seconds and death of the earthworm in around 30 seconds. In std. drug albendazole paralysis occurred in 6 min and death occur within 8 min. and in aqueous solution paralysis and death of worms occurred in around 45 min.
- According to all the extraction ethanolic extract of c. guianensis demonstrated high potency against earthworms.

CHAPTER- 5 RESULT AND DISSCUSION

RESULT AND DISSCUSION

1. Antimicrobial action of plant extract.

The extract of C. guianensis leaves was prepared by first washing the leaves thoroughly, followed by drying and grinding them into a fine powder. The resulting powder was subsequently mixed with various solvents, including ethanol, chloroform, and an aqueous solution, to prepare the plant extracts for antimicrobial testing.

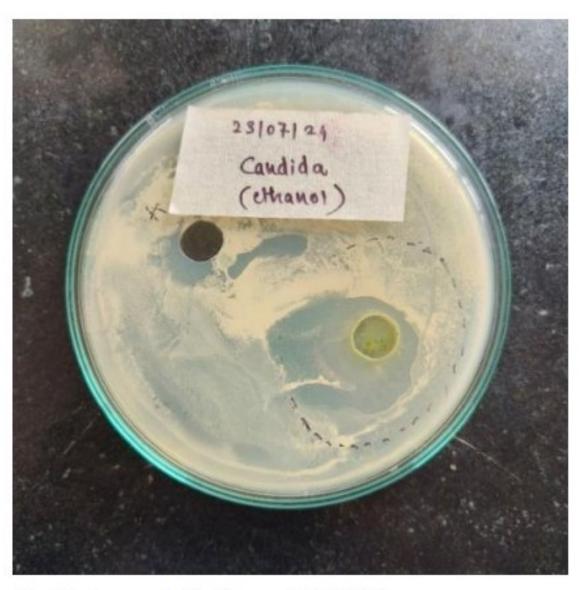
For the antimicrobial assay, the test organisms were inoculated onto sterile, solidified Mueller-Hinton agar plates using a sterile glass spreader. After spreading the microbial suspension evenly on the agar surface, wells were created in the agar plates and filled with the prepared plant extract (Gousia.s.k 2013). The plates were then stored in a refrigerator for 15 minutes to allow diffusion, followed by incubation at 37°C for 24 hours to assess microbial growth inhibition.

The C. guianensis plant extract demonstrated inhibitory activity against a range of bacterial pathogens, including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae, as well as the fungal species Candida albicans.

Table no. 1: Antimicrobial activity.

Sr.no	Organism	Control	Staph aureus	E. coli	P. aeruginosa	K. pneumonia	Candid albicans
1.	Ethanol (wet leaves)	;; = -	17mm	15mm	19mm	12mm	45mm
2.	Chloroform	-	_	= 8	_	_	
3.	Aqueous (dry leaves)	•	14mm	18mm	10mm	5mm	_

Antimicrobial activity of Couroupita guianensis



1) Photograph 2. Zone of inhibition



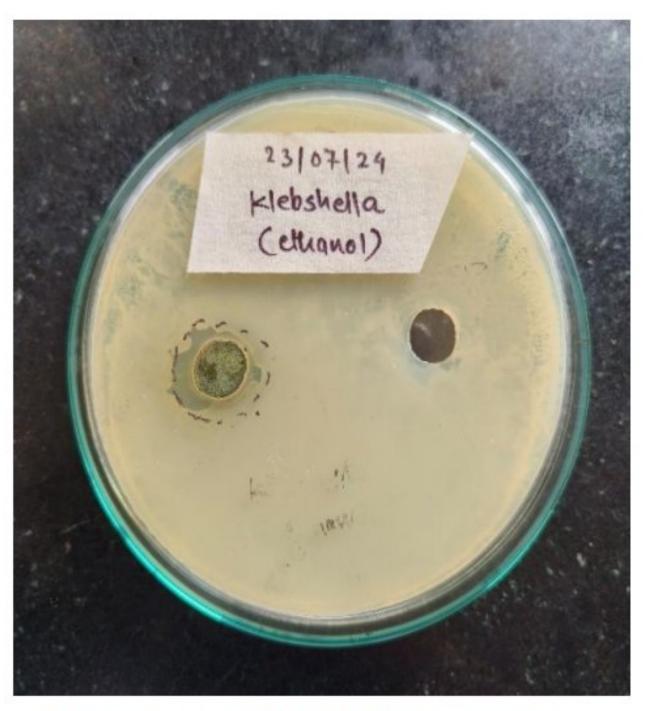
Photograph 3 zone of pseudomonas (wet leaves)



photograph 4 zone of s. aureus (wet leaves)



Photograph 5 zone of E. coli (wet leaves)



Photograph 6 zone of klebsiella (wet leaves)



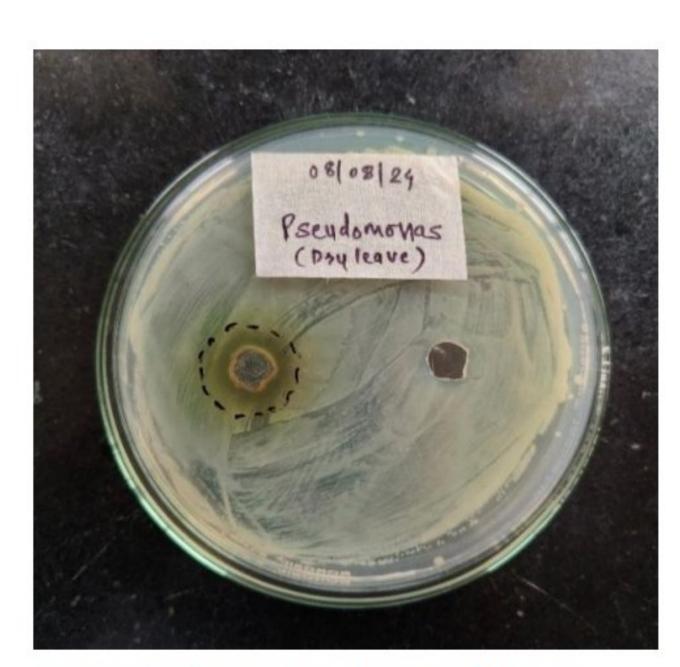
Photograph 7 zone of s. aureus (dry leaves)



Photograph 8 zone of E. coli (dry leaves)



Photograph 9 zone of klebsiella (dry leaves)



Photograph 10 zone of pseudomonas (dry leaves)

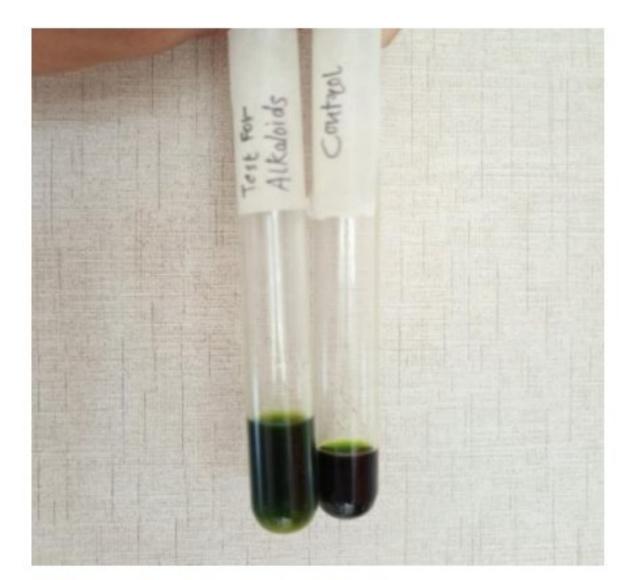
Phytochemical analysis of C. guianensis

The aqueous and ethanolic extracts of *Couroupita guianensis* were subjected to qualitative analysis to identify the presence of various phytochemical compounds. Standard tests were conducted to detect common phytochemicals, including alkaloids, tannins, carbohydrates, glucosides, phenols, and proteins, in the plant extracts.

Table no. 2: phytochemical analysis

Sr no.	Constituents	Methods	Result
1.	Alkaloids	Mayers test	+
2.	Proteins	Biuret test	+
3.	Phenols	Ferric chloride test	+
4.	Terpenoids	Salkowski test	+
5.	Steroids	Salkowski test	+
6.	Glycoside	Keller killani test	
7.	Tannins	Gelatine test	+

Phytochemical analysis



Photograph 11 test for alkaloids



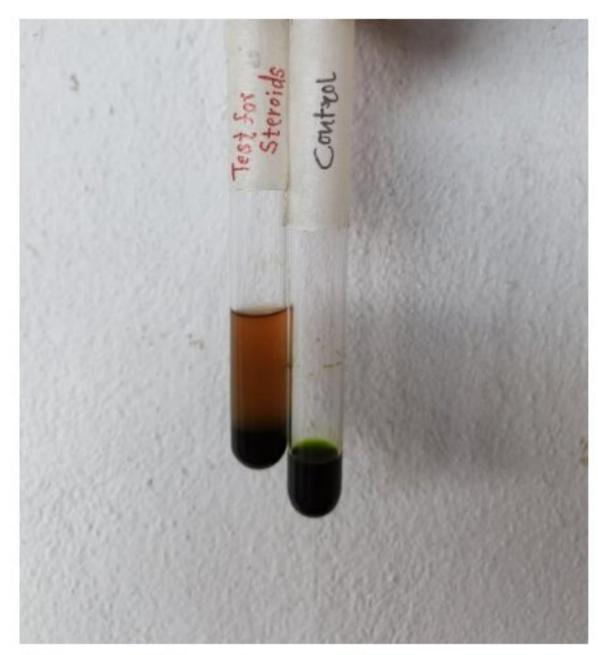
Photograph 12 test for protein



Photograph 13 test for phenol



photograph 14 test for terpenoids



Photograph 15 test for steroid



Photograph 16 test for glycosides



Photograph 17 test for tannins

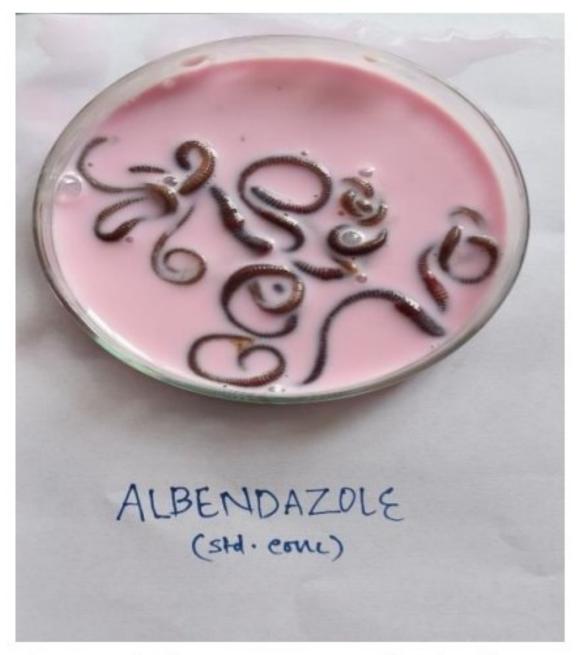
Anthelmintic activity

The aqueous and ethanolic extracts of *Couroupita guianensis* were subjected to qualitative analysis to identify the anthelmintic activity against various phytochemical compounds. Standard tests were conducted to detect the activity including standard albendazole syrup, ethanol extract and aqueous extract(Suresh.m.2017).

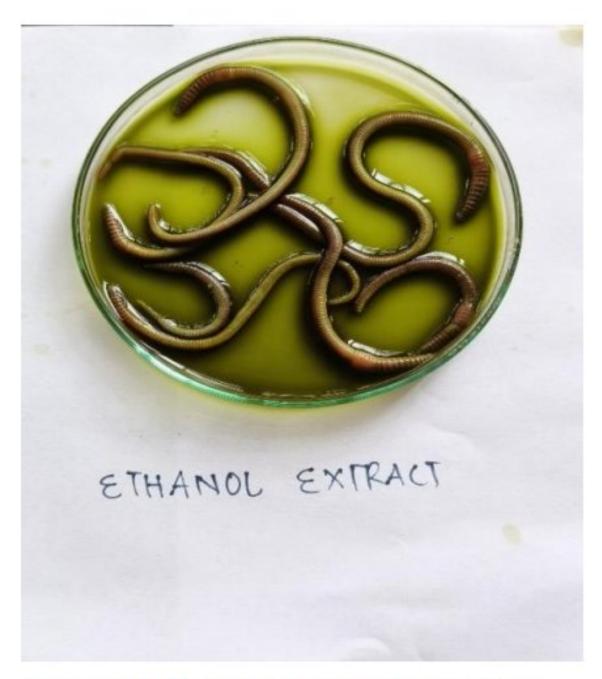
Table no. 3: Anthelminthic activity

Sr.no	Solvents	Time of paralysis	Time of death
1.	Albendazole	6 minutes	8 minutes
2.	Ethanol extract	25 seconds	30 seconds
3.	Aqueous extract	45 minutes	60 minutes

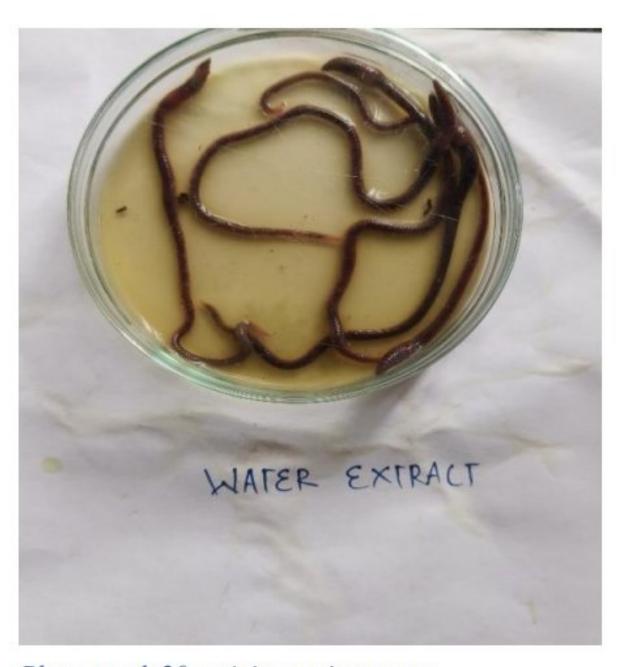
Antihelminth activity



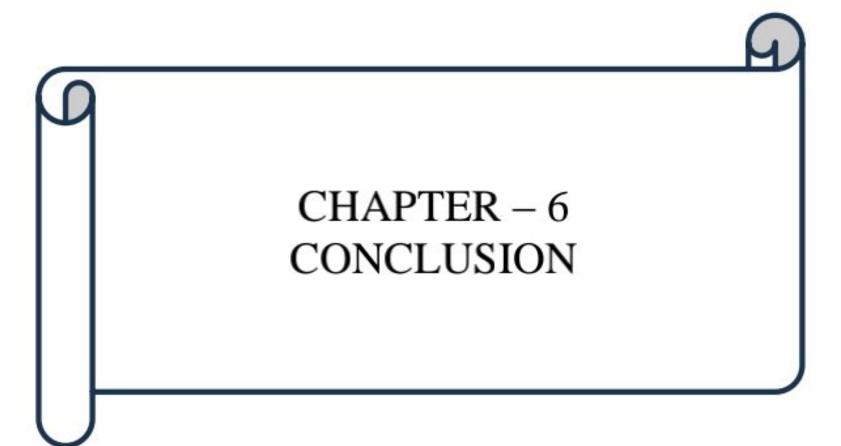
photograph 18 activity against albendazole



photograph 19 activity against ethanol extract



Photograph 20 activity against water



CONCLUSION

An extensive literature review indicates that Couroupita guianensis is a significant medicinal plant with a broad pharmacological profile. The plant contains a variety of chemical constituents, which contribute to its diverse pharmacological and therapeutic properties. Key active compounds identified in Couroupita guianensis include steroids, glycosides, carbohydrates, Couroupita A, Couroupita B, isatin, triterpenoids, eugenol, linoleic acid, neroli, trypanothione, linalool, phenolic resins, and dyes. These constituents collectively underline the plant's potential as a valuable resource for future scientific research and medicinal applications, offering promising prospects for human health.

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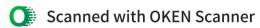
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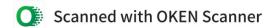
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