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IN-VITRO PHARMACOLOGICAL EVALUATION OF VARIOUS PARTS OF *COUROUPITA GUIANENSIS*

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ABSTRACT

The discovery of drug-resistant microbial species has intensified the search for plant-based therapeutic alternatives to synthetic antimicrobial compounds. Over the past few decades, the traditionally used medicinal plant *Couroupita guianensis* Aubl has been reported for its diverse therapeutic actions. This work highlighted the antibacterial, antifungal, and antioxidant properties of leaves, flowers, and fruits using successive solvent extractions and *in vitro* assay methods. By using solvent extraction, the powdered extracts were prepared and tested for phytochemical screening. These results confirm the presence of alkaloids, flavonoids, tannins, glycosides, terpenoids, phenols, and saponins. Antibacterial and antifungal activitieswereperformed using the agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Salmonella enterica*, with extracts showing concentration-dependent inhibition zones. The antibacterial activity was evaluated using the *in vitro* well plate method, antifungal activity by the well diffusion method, antioxidant activity by the DPPH and hydrogen peroxide scavenging assays, anti-inflammatory activity by the *in vitro* HRBC membrane stabilization method, and anti-arthritic activity by the protein denaturation method. The pharmacological results conclude that aqueous extracts showed less activity than chloroform extracts. The antioxidant activity was determined using the DPPH and H_2O_2 scavenging assays, and all plant extracts showed significant activity. These results support that *C. guianensis*is a valuable source for a natural therapeutic candidate.

KEYWORDS: Pharmacological activities, Extracts, Solvent extraction, and *Couroupita guianensis*, Soxhlet apparatus.

1. INTRODUCTION

In the past few decades, all over the world, infectious diseases have been a major crisis for humans. The scope of microbial therapy is to eradicate or inhibit the infecting species without harming the host. This is can be accomplished through the use of antimicrobial drugs.[1,2,4,7,9,11,12,13,14,18,21,22,24] The terminology surrounding the drugs used to treat infections is complex; a strict definition of the term antibiotic is that it is a substance produced by one living organism that kills or inhibits the growth of another. The term "antibiotic" originally referred exclusively to formulations derived from living organisms; however, it has evolved to encompass synthetic drugs as well.Antibacterial agents can be additionally classified into bactericidal agents, which eradicate bacteria, and bacteriostatic agents, which decrease bacterial growth.

Natural products[15,16,20], when compared to commercial antibiotics, are more effective with fewer side effects[5,6] The antimicrobial activity of *Couroupita guianensis*, including its leaf, flower, and fruit[17,19], is being investigated as an alternative source for producing new drugs to combat the dangers of drug-resistant and multidrug-resistant pathogens. Over the past few decades, these phytochemicals have been shown to have significantaction on long-term human health when consumed and can be used to efficiently treat variousdisorders.[3,8,10,23]

*Couroupita guianensis*Aubl., also known as the Cannonball tree, Sal tree, or Ayauma tree. Leaves of *C. guianensis*is widely used as analgesics by the rural population in Brazil. In various skin diseases, the Juice extracted from the leaves of this plant is used, and the shamans of South America have also utilized it. Moreover, the diverse parts of the *Couroupita guianensis* tree are crafted into infusions and teas that address a range of health issues, including hypertension, tumors, pain, and inflammation. These flowers are used to treat colds, intestinal gas formation, and stomachaches; the fruit pulp of *Couroupita guianensis* is used to disinfect wounds.

In this study, the antimicrobial activityof *Couroupita guianensis*, a species, and its various parts are examined. The primary objective of this study is to ascertain the antimicrobial action through an Ethnopharmacological survey. By highlighting the significance of this study, we aim to demonstrate the potential of *Couroupita guianensis* as a valuable resource in combating bacterial infections.

2. MATERIALS AND METHODS

2.1 MATERIALS

*Couroupita guianensis*Aubl (Cannonball tree, Sal tree, Ayauma tree) Leaves juice**,** flowers powder, fruits pulp.

Table 1: Antibiotics used in standards.

Organisms	Antibiotic used as a standard
Streptococcus aureus (Gram-positive)	Penicillin
E. coli (Gram-negative)	Doxycycline

2.2 METHODOLOGY

2.2.1 METHODOLOGY OF LEAF

We carefully gathered the plant and lovingly washed its leaves with sterile distilled water to ensure they were free from any dirt or impurities. They were then shade-dried at room temperature for about 10 days. The shade-dried leaves were pulverized in a mixer grinder to form a fine powder and then passed through a mesh with a 100-micron mesh size.

Fig. 1: *Couroupita guianensis* leaf and its powder.

2.2.2. Preparation of extracts by successive solvent extraction

The finely powdered leaf drug of *Couroupita guianensis*, approximately 80 g, was extracted with chloroform (50-55°C) for 72 hours using the continuous hot percolation method with a Soxhlet apparatus. Then it was evaporated to form a dry mass of chloroform extract.

An aqueous extract is prepared by dissolving 500 mL of distilled water in 500 g of finely powdered leaf material of *Couroupita guianensis*and is kept for 36 hours. Then it filtered and evaporated.

2.2.3.Anti-bacterial activity: The prepared leaf extract of *Couroupita guianensis*was tested for anti-bacterial activity.

2.2.4.Anti-fungal activity: The prepared leaf extract of *Couroupita guianensis*was tested for anti-fungal activity.

2.3. METHODOLOGY FOR FLOWER

The fresh leaves and flowers of *Couroupita guianensis* were collected. They were washed separately to remove foreign matter, then dried by the shade-drying method. The drying of fresh leaves was carried out under natural airflow, and surrounding temperature (25°C) for 240 hours (10 days), and the flowers were dried under natural airflow for 120 hours (5 days). After drying, the leaves and flowers were ground into fine powders using a grinder, and the powders were stored at room temperature.

Fig. 2: Flower of *Couroupita guianensis*and its powder.

2.3.1. Extraction: The dried flower (250g) of *C. guianensis* sample was extracted with methanol (500ml) using a Soxhlet apparatus. Then the methanol extract was concentrated and weighed (10g). The dry extract was stored at 40°C until use. Qualitative phytochemical screening of *Couroupita guianensis* flower extract: Phytochemical screening of methanol extract.

2.3.2. Determination of antioxidant activity by DPPH-scavenging assay: The prepared flower extract of *Couroupita guianensis* was tested for antioxidant activity by DPPH-scavenging assay method.

2.3.3. Determination of antioxidant activity by hydrogen peroxide scavenging assay: The prepared flower extract of *Couroupita guianensis* was tested for antioxidant activity by hydrogen peroxide scavenging assay method.

2.3.4. Anti-bacterial Activity: The prepared flower extract of *Couroupita guianensis* was tested for anti-bacterial activity.

2.4. METHODOLOGY FOR FRUIT

The fruits of *Couroupita guianensis*Aubl. are collected from the premises. The plant was identified with the help of Floras and, in consultation with the experts in angiosperm taxonomy. Collected fruit is subjected to different processes for studying its activity.

Fig. 3: Fruit and its powder of *couroupita guianensis.*

2.4.1. Anti-inflammatory activity: The prepared fruit extract of *Couroupita guianensis* was tested for anti-inflammatory activity.

2.4.2. Anti-arthritic activity: The prepared fruit extract of *Couroupita guianensis* was tested for anti-arthritic activity.

3. RESULTS AND DISCUSSION

3.1. Results and Discussion for Leaf

Table 2: Qualitative analysis of bioactive compounds in different solvent extracts of *couroupita guainensis* leaves.

Test name	Chloroform extract	Aqueous extract
Mayers	++	+
Wagers	++	+
Dragendroffs	++	+
Tannins	+	+
Glycosides	++	++
Sterols	++	+
Resins	++	+++
Phenols	+	+
Anthraquinones	++	+
Carbohydrates	++	++
Cardiac glycosides	-	-
Steroids	+	+
Terpenoids	++	++
Alkane reagent test	+	+

Table 3: Inhibition zone diameter of different extracts of *couroupita guianensis* against.

ORGANISM				ORGANISM			
Staphylococcus Aureus				*E. Coli*			
Chloroform		Aqueous		Chloroform		Aqueous	
Concentration (mg/ml)	Zone of Inhibition (cm)	Concentration (mg/ml)	Zone of inhibition (cm)	Concentration (mg/ml)	Zone of inhibition (cm)	Concentration (mg/ml)	Zone of inhibition (cm)
50	0.5	50	-	50	1.2	50	-
100	1.5	100	0.5	100	1.85	100	0.3
150	1.8	150	0.95	150	2.2	150	0.5
200	2.2	200	1.2	200	2.67	200	0.96

Pencillin 10ug/ml	3.5	Pencillin 10ug/ml	3.5	Doxycylin 10ug/ml	2.8	Doxycylin 10ug/ml	2.8

different fungus. (Effect of chloroform extract on selected bacteria)

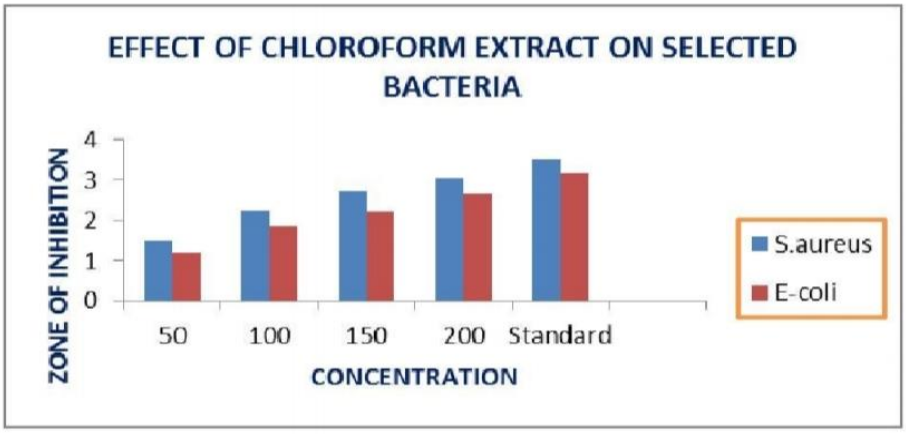

Fig.4: Effect of chloroform extract on selected bacteria.

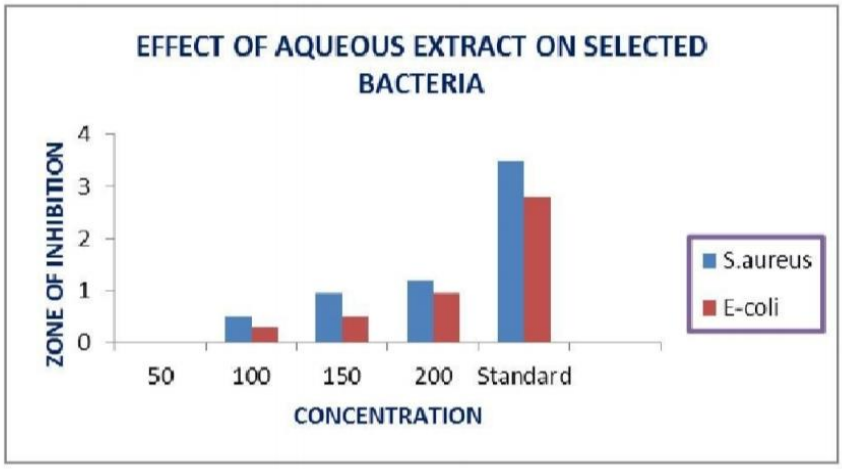

Fig.5: Effect of aqueous extract on selected bacteria.

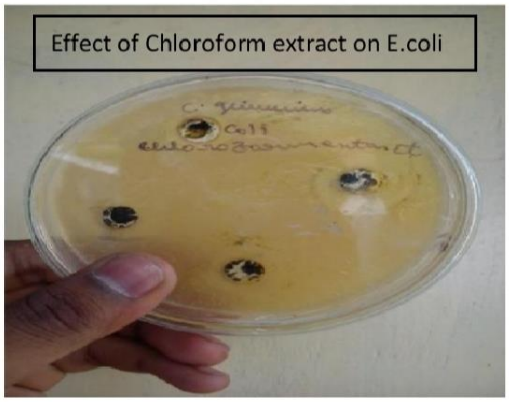

Fig.6: Standard and control of selected bacteria Fig.: Effect of chloroform on *E. coli.*

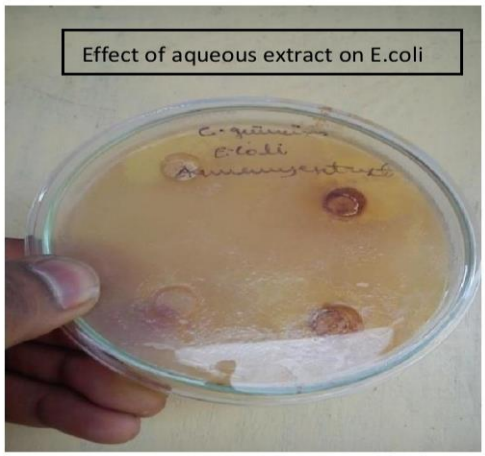

Fig.7: Effect of aqueous extract on *E.coli.*

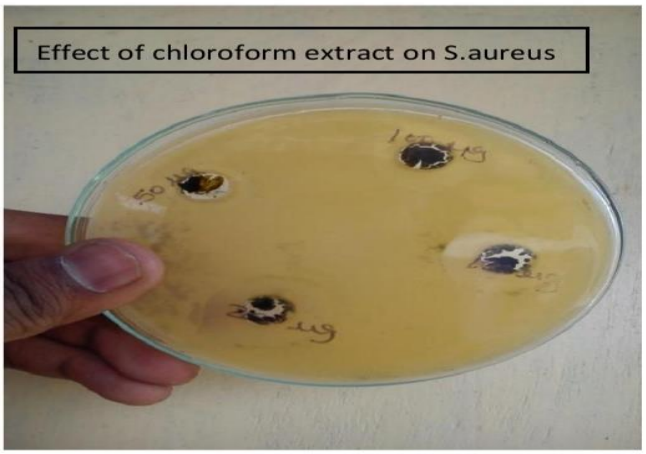

Fig.:Effect of chloroform extract on S. *aureus.*

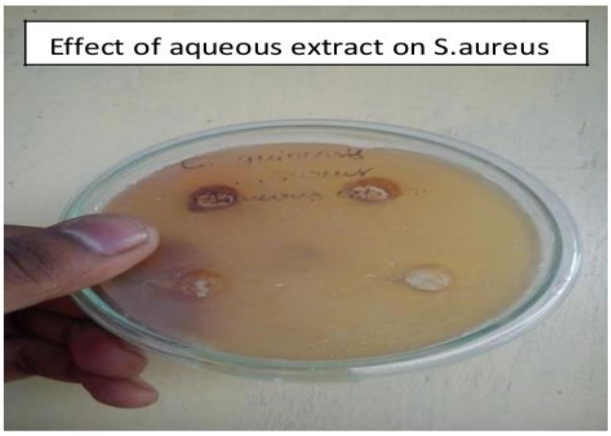

Fig.8: Effect of aqueous extract on S. *aureus.*

4. RESULTS AND DISCUSSION

The various extracts (Leaf, Flower, and Fruit) of *Couroupitaguainensis* were screened for their antimicrobial and pharmacological activities using chloroform and water. The study used the agar well diffusion method to test against pathogenic bacterial species *Staphylococcus aureus* and *E. coli*. According to the results above, the chloroform extract demonstrated significantly greater antibacterial activity than the aqueous extract. The observed zone of inhibition was measured 2.2 mm against *Staphylococcus aureus* and 2.67 mm against *E. coli*, whereas the aqueous extract showed zones of 1.2 mm and 0.96 mm, respectively.The chloroform extract of *Couroupita guianensis* showed greater activity against bacteria, including*Staphylococcus aureus*. Preliminary phytochemical analysis of chloroform extract & aqueous extract showed the presence of flavonoids, glycosides, alkaloids, steroids, triterpenoids, whereas the aqueous extract revealed the tannins, glycosides & alkaloids as active phytochemical constituents.

Whereas the phytochemical screening of the Flower extracts of *Couroupita guianensis* confirmed the presence of alkaloids, phenols, flavonoids and reducing sugars. The extracts demonstrated a remarkable increase in antibacterial activity with rising concentrations (µg/ml). In comparison with standard drugs, the results revealed that the extracts were more effective against the bacterial activity of Bacillus subtilis and Salmonella enterica than against E. coli and Staphylococcus aureus. The growth inhibition zone measured ranged from 14 to 24 mm for all the sensitive bacteria (Image 1). The results show that the extracts of *Couroupita guianensis* were found to be more effective against all the microbes tested.The present study concludes that the flowers of Couroupita guianensis contain high antioxidant and antibacterial properties. The presence of phytochemicals, including reducing sugars, proteins, alkaloids, phenols, tannins, saponins, glycosides, flavonoids, terpenes, and steroids. The plant extract is certainly effective in managing the disease caused by these organisms. There is a need for further investigation of the plant to identify and isolate its active antibacterials.

The result of phytochemical analysis of *Couroupita guianensis*Aubl. This involved the qualitative determination of flavonoids, tannins, terpenoids, steroids, cardiac glycosides, alkaloids, phenols, and carbohydrates. Amino acids and sterols were found to be absent in the sample through preliminary investigation. The free radical scavenging activity of the solvent was

assured by the ABTS assay. The antimicrobial activity of leaf, flower, and fruit extracts was evaluated using the agar well diffusion method against Staphylococcus aureus and E. coli. The outcomes of the antimicrobial activity of *C.guianensis* plant extracts suggest that the plant may have potential for the discovery of new antimicrobial components. The present study concludes that CgFexhibits a more pronounced antibacterial activity than CgL against the standard.

Table 4: Phytochemical screening of flower extracts.

S.NO	TEST FOR	RESULT
1.	Carbohydrates	+
2.	Proteins	-
3.	Alkaloids	+
4.	Phenols	+
5.	Tannins	+
6.	Saponins	+
7.	Glycosides	+
8.	Flavonoids	+
9.	Terpenes	+
10.	Steroids	-

Table 5: antibacterial activity of the flower extract of *couroupita guianensis* by agar well diffusion method.

S.no	TYPE OF STRAIN	Zone of inhibition(mm)			Standard (mm)
		(100µg/ml)	(200µg/ml)	(300µg/ml)	
1	*Escherichia coli*	14.3±0.24	17.1±0.32	22.4±0.28	26
2	*Staphylococcus aureus*	15.3±0.24	18.2±0.32	23.2±0.52	26

Table 6: Concentration and percentage inhibition of fruit.

SAMPLE NAME	CONCENTRATION(mg)	PERCENTAGE OF INHIBITION
Fruit	10mg	11.96%
	20mg	31.70 %
	30mg	51.43%

Fig.9: Effect of chloroform and aqueous extract by *e.coli.*

Fig.10: Effect of chloroform and aqueous extract by *s. Aureus.*

5. CONCLUSION

The antimicrobial activity of the different extracts of this cannonball tree was assessed against various human pathogenic bacteria. The present study was analysed with the aim of establishing standards that could aid in detecting the authenticity of this vital medicinal plant.

The numerical standards reported in this work could be useful for compiling a suitable monograph of *Couroupita guianensis*. The results obtained from this revealed that the plants contained bioactive agents associated with antimicrobial properties.

The wide spectrum of antifungal activity of *Couroupita guianensis* extracts has been documented. From the obtained results, it can be concluded that although chloroform in itself exhibits antifungal activity comparing with aqueous extract, the chloroform extract of *Couroupita guianensis* displays synergistic activity. Since *Couroupita guianensis* is easily available and well-tolerated, it can be incorporated into medications for tropical antifungal therapy. However, further studies are needed to assess its incorporation into oral preparations, safety, and cost-effectiveness.

The present study concludes that the flowers of *Couroupita guianensis* contain high antioxidant and antibacterial properties. The presence of phytochemicals, including reducing sugars, proteins, alkaloids, phenols, tannins, saponins, glycosides, flavonoids, terpenes, and steroids. The plant extract is certainly effective in managing the disease caused by these organisms. There is a need for further investigation of the plant to identify and isolate its active antibacterials.

The outcomes of the antimicrobial activity of C. guianensis plant extracts suggest that the plant may have potential for the discovery of new antimicrobial components. The present study concludes that CgFexhibits a more pronounced antibacterial activity than CgL against the standard. Plant-based antimicrobials have fewer side effects and are a boon to the development of traditional plant systems. Hence, the antimicrobial activity of the plant extract of Couroupita guianensis was assessed against human pathogenic bacteria, including *S. aureus* and *E. coli*. The results show that the extract exhibits greater antimicrobial activity than the standard. This study provides information that may sparkresearchers' interest in exploring such natural resources.

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