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Formulation And Evaluation Of Anti-Bacterial Herbal Gel Of The Couroupita Guianensis Extract

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ABSTRACT

Herbs or plants with medical properties, are the source of herbal remedies. There are various groupings within the plant kingdoms; however, botanical classification is not provided in this section. Conversely, there are four types of herbal plants: climbers, woody perennials, perennials and biennials, trees, and shrubs. This page only discusses blooming plants; fungus, fern, moss, an

Synthetic drugs based on chemicals mainly obtained from plants are frequently used in medical technologies. Therefore, the identification of the plants comes from Man's long-standing practice of medicinal herbalist, whether the plants are used in their entirety, in extract, or in synthetic compounds.

Anytime bacteria evolve in reaction to antibiotic treatment, antibiotic resistance develops. Antibiotic-resistant bacteria develop resistance to them, not people or animals. Living things are infected by these bacteria, and treating their illnesses is more challenging than treating infections brought on by non-resistant microbes.

Antibiotic overuse raises mortality rates, lengthens hospital stays, and raises medical expenses. Today, varied approaches to the administration and usage of antibiotics are required globally. Antibiotic resistance can be mitigated by vaccination, hand washing, sexual health, and good food hygiene, even in the case that new medications are created.

A gel is a system that is solid or semisolid and has a minimum of two elements. The condensed mass encircles and penetrates the liquid. While having a more solid than liquid consistency, gels and jellies are made of a very small number of particles dispersed throughout a sizable volume of liquid. The dermal structure that gives gel and jelly their solid-like properties sets them apart from one other.

The name "natural herbal gels" refers to the place where the ingredients used to make the gel originated. Developing an appropriate formula using concentrated extracts of medicinal plants can be helpful in reducing the health issues and disease associated with recurrent herpes labials because herbal medicines are more widely accepted globally due to their minimal side effects and lower cost.

Key word- material and method, application, evolutions, result and conclusion.

Introduction

Understanding the importance of these resources, the area integrates different social and economic interests while putting the preservation of its distinct ecology as its top priority in order to promote their conservation. Due to its extraordinary chemical variety, the Legal Amazon's remarkable biodiversity places it at the forefront of research on herbal medicine and presents many opportunities for the discovery of novel medications [1]. Because phytotherapy offers natural compounds with better biocompatibility, less toxicity, and scientifically demonstrated therapeutic activity than traditional medications, it has become more well-known in the dental community.

The plant species known as Couroupita guianensis Aubl., or "abricó de macaco" in Brazil, is indigenous to the Legal Amazon [2]. This plant's leaves, flowers, fruits, roots, stems, and seeds have all been connected to a number of health advantages. Treating malaria is one of these attributes.[3], control blood pressure, have antibacterial and analgesic qualities [4-5], and are healing and anti-inflammatory [4-6]. Additional research has revealed the presence of numerous additional chemicals in C. Guianensis, including volatile compounds, flavonoids, alkaloids, saponins, and quercetins [6]. Microbiologists have studied the plant Couroupita guianensis Aubl. in the past, but little is known about its capacity to control oral harmful microorganisms. The goal of this work was to ascertain the phytochemical and toxicological characteristics of leaf extracts that were extracted from Couroupita guianensis Aubl. Furthermore, the investigation concentrated on microbes commonly detected in the oral cavity to evaluate the antibacterial and antioxidant characteristics of these extracts.

Methodology:

Collection of the sample:

Plant Constituents

In specific, the plant material was found in 48°19′56″ W and 10°11′14″ S. The leaves were meticulously numbered and tagged after they were collected to help with future research and serve as a reference. For the herbarium collection, these leaves have been designated.

Figure 1:- Leaf couroupita guianensis

1. Extracts are prepared by sequential solvent extraction

A soxhlet apparatus and a continuous hot percolation process lasting 72 hours were used to extract 80g of finely powdered Couroupita guianensis leaf medication at a temperature of 50–55 degrees Celsius. The bulk of the chloroform extract was then dryly produced by evaporating it. An aqueous extract of 500g of finely ground couroupita guianensis leaf medicine is stored for 36 hours after being mixed with 500ml of distilled water.After that, it is evaporated and filtered.A Soxhlet device and a continuous hot percolation procedure conducted for 72 hours at 50–55 degrees Celsius were used to extract 80g of finely crushed leaf medicine from Couroupita guianensis.The bulk of the chloroform extract was then dried by evaporating it.Dissolving 500ml of distilled water in 500g yields an aqueous extract.

2.Phytochemical Screening

The principal classes of secondary metabolites contained in the extracts were determined by phytochemical screening. A qualitative test based on particular chemical reactions that produced hue shifts or precipitation was part of the screening process. Subsequent classes of secondary metabolites that required identification were alkaloids, quinones, triterpenoids, flavonoids, tannins, and phytosterols.

Table 1:- Phytochemical Screening

Tests	Methods	Expected observation	Inferences
Alkaloids	1mLsample and 3mLMayer'sreagent.Incubation at 60°Cfor 30minutes.	Formation of creamish ppt	Alkaloids presence.
Flavonoids	NaOH and HCl were combined with 1 milliliter of the extract.	The color less solution turns yellow	Show the existence of flavonoids.
Tannins	1% lead acetate was added in1ml extract.	Formation of yellowish ppt	Existence of tannins.
Carbohydrate	1 mL FA reagent and1mL FB reagent and 1 mL extract were added and then incubated at 80°Cfor30min.	Red precipitate	Existence of carbohydrates
Steroid	Measured amounts of sulphuric acid were added to the test tube's sidewalls after 1 mL of extraction was combined with 10 mL of chloroform.	In addition to the walls of the tubes turning yellow, the player turns crimson.	Steroid is present
Saponins	One milliliter of extraction was added with five milliliters. Allowed for vigorously shaking.	Froth formation up to one cm	Presence of saponins.
Fatty acids	One milliliter of extraction was combined with ten milliliter so feather and permitted to dissolve on filter paper. The filter paper had been dried out.	Existence of transparency.	Fatty acid present

3. Evaluation of prepared herbal gel:

- **Colour:** White and black backdrops were used to assess the compositions' color.
- **Odour:** Dissolving a little amount of gel in alcohol allowed us to assess the aroma of the gels.
- **Uniformity**: The texture of the gel was evaluated by applying it to the outer layer of the skin.
- **Greasiness:** It was possible to observe how greasy the compositions were after applying the solutions to the skin.
- **Homogeneity** was assessed by visually inspecting all of the prepared gels after they had solidified in the container. An examination was conducted to determine the absence of any aggregation and how they appeared.
- **pH Measurement:** The pH levels of several ointment formulations will be measured using a digital pH metre. One gramme of ointment and one hundred milliliters of distilled water should be combinedLet the mixture alone for two hours without stirring. The pH of each formulation will be measured three times, and the mean value will be determined.
- **Extrude ability study:** Extrudability was determined by measuring the percentage of gels that extruded under finger

pressure. As the amount extruded increased, extrudability improved.

- **Spreadibility:** Following their application to a human's skin, the effects of the herbal gels were evaluated visually in table [16].

Evaluation of herbal gel:

4. Antibacterial Activity

An in vitro well diffusion method was employed to assess the antibacterial activity of the previously stated leaf extracts against the widely used antibiotic Penicillin (10 mg/ml). The test organism was grown in a grass culture using nutrient agar. Following a brief period of time, four wells were excised from the contaminated plates at the appropriate distance using a well cutter. After every phase of well cutting, alcohol was used to completely clean the well cutter. Next, based on a predetermined amount, each well was treated with a progressively higher concentration of the plant extract (100µl). For twenty-four hours, the bacterial plates were incubated at 37˚C. By measuring the zone of inhibition's diameter, the extract's activity was ascertained. An acquired bacterial strain was utilized [17].

5. Antifungal Activity

The antifungal activity of the leaf extracts against the prescription medication Clotrimazole (10 mg/ml) was evaluated in vitro using the well diffusion method. The test organism was grown on Potato Dextrose Agar (PDA) in order to generate a lawn culture. within using a well cutter at the appropriate distance, four wells were made in the contaminated plates within a few minutes.

Next, using 100µl of each extract at progressively higher concentrations, the extracts were applied to each well in line with a predefined methodology. The plates containing the fungus were cultured at room temperature for 48 hours. To determine the activity of the extract, the diameters of the zone of inhibition were examined.

Both pototao dextrose agar (PH 5.5–6) and nutritive agar/broth (PH 7.4) can be used to test for antibacterial sensitivity. A sensitivity test is performed in vitro using samples that were recently infected. The 24 year old culture is the source of the bacteria that have been filtered in the columns.

The density of the test-injected colony forming units employed for testing is about 105 cells/ml.The length of the incubation period and the temperature: Antibacterial sensitivity test findings are always read following an 18–24 hour incubation period at 370C.

The study employed four human pathogenic microorganisms, namely Aspergillus niger, Candida albicans, Escherichia coli, and Staphylococcus aureus, to quantify the antibiotic activity. Two fungal strains and gram positive and gram negative bacterial strains were discovered in the laboratory [16-17].

Table 2:-A tabulation of the strain's name and kind

S.NO.	STRAIN NAME	TYPE
A	*Candida albicans*	Pathogenicity in humans
B	*Aspergillus niger*	Pathogenicity in humans
C	*Streptococcus aureus(Gram +VE)*	Pathogenicity in humans
D	*Escherichia coli (Gram -VE)*	Pathogenicity in humans

Table 3:-Bacterial nutrient agar (pH 7.4)

Contents	Amount
Peptone	5grams
Beef Extract	3grams
NaCl	5 grams
Agar	20 grams
Dist. water	1000ml

Table 4:-Fungi on potato dextrose agar

Contents	Amount
Dextrose	15grams
Peeled Potato	100 grams
Agar	15 grams
Dist. water	1000ml
pH	5.6

STERILIZATION AND MEDIUM PREPARATION

After combining 35 grams of agar medium with one liter of distilled water, the mixture was sealed in a screw-capped container and autoclaved for 15 minutes at 121°C. The medium was then put into sterilized, 90mm agar plates and left to set. The agar plates were incubated at 37°C to guarantee sterility. If, after a day, the plates exhibited no evidence of development, they were considered sterile. The four human pathogenic bacteria used for normal culture in this study were Escherichia coli, Aspergillus niger, Staphylococcus aureus, and Candida albicans[18].

Plating the media

We used the previously outlined methods to perform the antibacterial activity screening. Finally, a wire loop was used to remove the microbe cultures from the culture plates. The cultures were then mixed individually with regular saline and stirred with a vortex mixer. Using the Pour Plate method, a loop fill was removed and aseptically added to the cooled, sterilized Nutrient agar medium. On the surface of the solid medium, wells formed that measured about 4 mm in diameter and 2.5 mm in depth. equipment used by a sterile borer. After the plates were turned over, markers were used to identify each well. A zone reader was used to measure the zone of inhibition after the plates were in for a full day, and the data were combined[19].

ANTIBIOTICS USED AS STANDARDS

Table 5:-Antibiotics used as standards

Organisms	Antibiotic used as Standard
Streptococcus aureus (gram +ve)	Penicillin
Escherichia coli (gram -ve)	Doxycycline
Aspergillus niger	Clotrimazole
Candida albicans	Clotrimazole

RESULTS AND DISCUSSION

1. Extracts are prepared by sequential solvent extraction:-

Table 6:- A qualitative examination of the bioactive elements discovered in the various Couroupita Guianensis leaves extracted using test

Test Name	Chloroform Extract	Aqueous Extract
Mayers's	+++	++
Wagner's	++	++
Dragendroff's	++	+
Tannins	+	+
Glycosides	++	++

Sterols	+++	++
Resins	++	+++
Phenols	+	++
Anthraquinones	++	+
Carbohydrates	+++	++
Cardiac Glycosides	-	-
Steriods	+	+
Terpenoids	++	++
Alkaline reagent test	+	++

2.Phytochemical Screening:-

Table 7:-Tests for various phyto-constituents Phytochemical analysis of ethanolic extract

Secondary metabolite	***Couroupita guianensis***
Tannins	+
Flavonoids	+
Alkaloids	+
Carbohydrates	+
Steroid	+
Saponins	+
Fatty acids	+

Parameters	**Day -0**			**Day-14**			**Day 28**		
Physical Parameters	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
pH	6.9	6.9	6.8	6.9	6.8	6.9	6.9	6.9	6.8
Colour	Pale yellow	Strong yellow	Strong yellow	Pale yellow	Strong yellow	Strong yellow	Pale yellow	Strong yellow	Strong yellow
Spreadability	+++	+++	+++	+++	+++	+++	+++	+++	+++
Uniformity	Significance	Significance	Significance	Significance	Significance	Significance	Significance	Significance	Significance

Greasiness	No	No	No	No	No	No	No	No	No
Odour	Vinegary	Vinegary	Vinegary	Vinegary	Vinegary	Vinegary	Vinegary	Vinegary	Vinegary
Extrudability	+++	+++	+++	+++	+++	+++	+++	+++	+++
Homogeneity	++	++	++	++	++	++	++	++	++

Table 8:- Different Physical Parameters

4. Antibacterial Activity

Table 9:-Different Couroupita guianensis extracts' effects on certain microorganisms

Organism				**Organism**			
Streptococcus aureus (Gram +VE)				***Escherichia coli (Gram -VE)***			
CHLOROFORM		**WATER**		**CHLOROFORM**		**WATER**	
Conc.(mg/ml)	**ZOI (cm)**	**Conc.(mg/ml)**	**ZOI (cm)**	**Conc.(mg/ml)**	**ZOI (cm)**	**Conc.(mg/ml)**	**ZOI (cm)**
50	0.61	**50**	-	**50**	1.312	**50**	-
100	1.62	**100**	0.61	**100**	1.861	**100**	0.312
150	1.93	**150**	0.982	**150**	2.312	**150**	0.623
200	2.31	**200**	1.423	**200**	2.661	**200**	0.980
Penicillin (10µg/ml)	3.73	**Penicillin (10µg/ml)**	3.71	**Doxycycline (10µg/ml)**	2.90	**Doxycycline (10µg/ml)**	2.832

5. Antifungal Activity:-

Table 10:-Aspergillus niger and the effects of chloroform extract

Conc. (mg/ml)	**Day-1**	**Day-2**	**Day-3**	**Day-4**	**Day-5**	**Day-6**	**Day-7**
50	0.73	0.78	0.99	1.12	1.23	1.75	1.51
100	0.84	1.10	1.45	1.68	1.72	2.10	2.23
150	1.12	1.43	1.89	2.08	2.23	2.50	2.74
200	1.11	1.54	2.18	2.41	2.56	2.82	3
Chloroform	0.52	0.72	0.86	0.93	0.89	1.10	1.09
Clotrimazole (µg/ml)	1.36	2.06	2.56	2.84	3.03	3.21	3.7

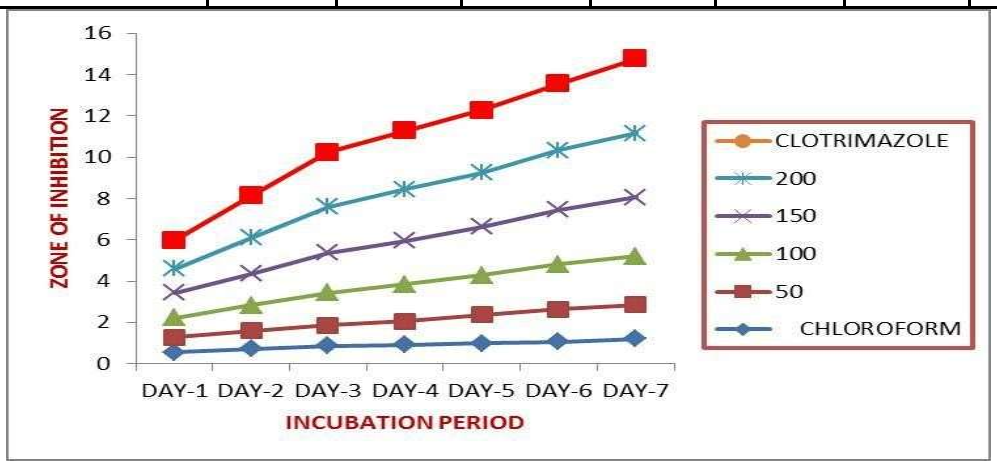
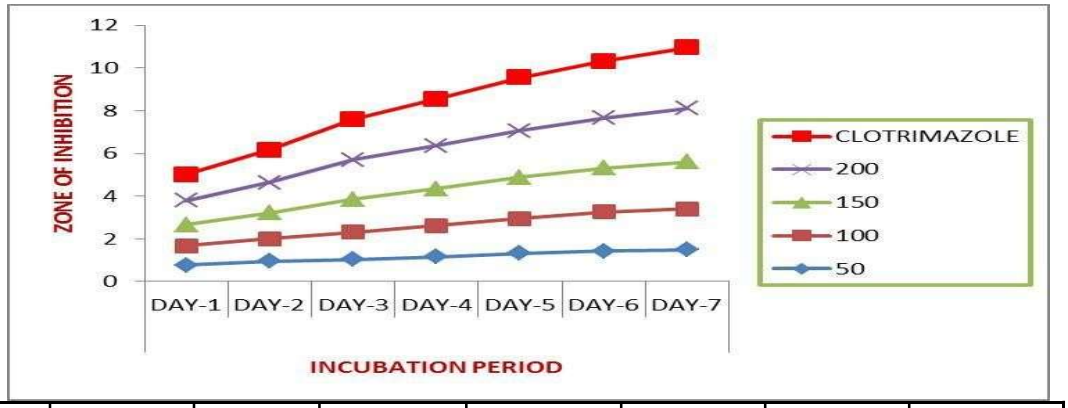


Figure 2:- Aspergillus niger and the effects of chloroform extract

Table 11:- Aqueous extract's effects on Aspergillus niger



Conc. (mg/ml)	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
50	0.79	0.96	1.05	1.13	1.30	1.39	1.7
100	0.8	1.07	1.30	1.42	1.60	1.80	1.91
150	0.96	1.22	1.55	1.73	1.90	2.09	2.21
200	1.17	1.40	1.88	2.08	2.16	2.36	2.45
Water	-	-	-	-	-	-	-
Clotrimazole (µg/ml)	1.21	1.54	1.92	2.18	2.43	2.67	2.84

Figure 3:- Aqueous extract's effects on Aspergillus niger

Table 12:-Candida albicans and the effects of chloroform extract

Conc. (mg/ml)	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
50	1.70	2.33	2.70	2.90	3.10	3.50	3.79
100	2.20	2.55	2.86	3.25	3.63	3.86	4.23
150	2.55	3.28	3.45	3.78	4.09	4.49	4.79
200	3.16	3.93	4.19	4.39	4.75	5.01	5.01
Control	0.75	0.85	0.88	0.96	1.06	1.23	1.41
Clotrimazole (µg/ml)	2.16	2.46	2.76	2.94	3.23	3.37	3.69

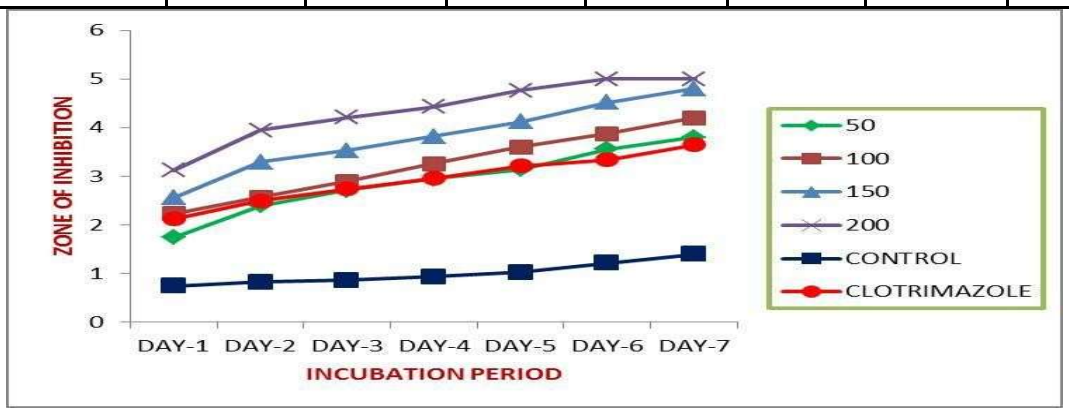
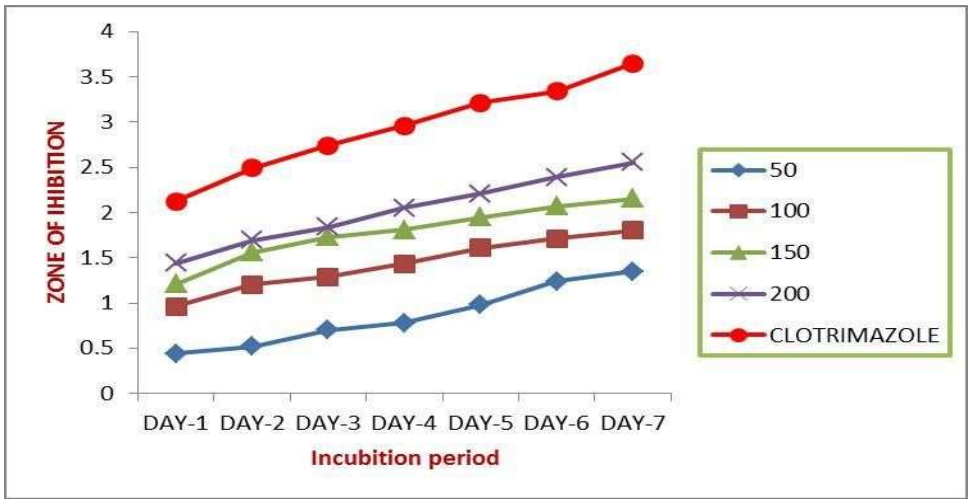


Figure 4:-Candida albicans and the effects of chloroform extract

Table 13:-Candida albicans and the effects of aqueous extract

Conc. (mg/ml)	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7
50	0.46	0.51	0.73	0.74	0.96	1.26	1.36
100	0.91	1.30	1.30	1.44	1.65	1.69	1.78



150	1.25	1.54	1.71	1.78	1.91	2.10	2.19
200	1.48	1.72	1.86	2.08	2.26	2.40	2.59
Water	-	-	-	-	-	-	-
Clotrimazole (µg/ml)	2.16	2.47	2.72	2.94	3.20	3.32	3.63

Figure 5:-Candida albicans and the effects of aqueous extract

Table 14:-Aspergillus niger and Candida albicans' responses to chloroform extract

Conc.(mg/ml)	*Aspergillus niger*	*Candida albicans*
50	**1.940 ± 0.351**	**2.8895 ± 0.702**
100	**1.7072 ± 0.427**	**3.2317**
150	**2.092 ± 0.5623**	**3.8157 5**
200	**2.3145± 0.6616**	**4.25514 ± 0.814**
Chloroform	**0.9112 ± 0.23214**	**0.99874 ± 0.247**
Clotrimazole	**2.7245 ± 0.7456**	**2.9122± 0.2145**

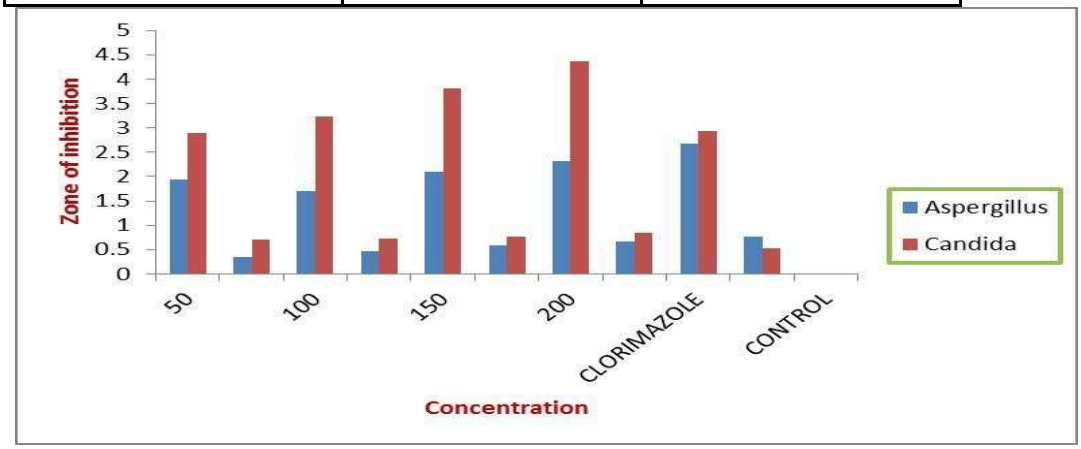


Figure 6:-Aspergillus niger and Candida albicans' responses to chloroform extract

Table 15:-Impact of aqueous extract on Aspergillus niger and Candida albicans

Conc.(mg/ml)	*Aspergillus niger*	*Candida albicans*
50	**1.16541 ± 0.2852**	**0.865423 ± 0.34751**
100	**1.42135 ± 0.38542**	**1.43254 ± 0.3123**
150	**1.6714 ± 0.459**	**1.75849 ± 0.31423**
200	**1.8345 ± 049452**	**2.0324 ± 0.34587**
Water	**-**	**-**
Clotrimazole	**3.6477 ± 0.678**	**2.99 ± 0.5325**

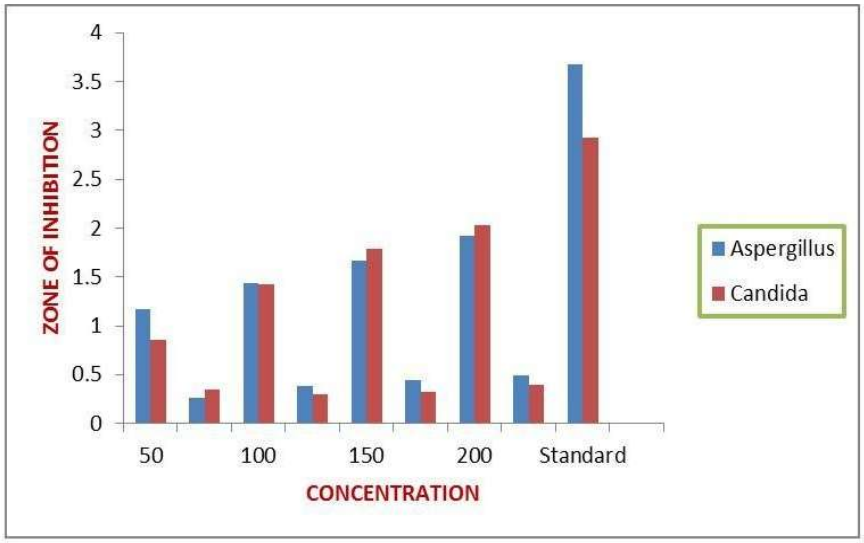


Figure 7:-Impact of aqueous extract on Aspergillus niger and Candida albicans

Discussion

Researchers looked at the pharmacological and antibacterial properties of leaf extracts from Couroupita guianensis. Water and chloroform were the solvents utilized to remove the leaves. The extract was evaluated using the Agar well diffusion method against a range of bacterial and fungal pathogens, including Staphylococcus aureus, Escherichia coli, and Candida albicans, that may cause infectious illnesses. Greater efficacy against fungi such as Candida was demonstrated by the chloroform extract of Couroupita guianensis.

At 50, 100, 150, and 200 mg/ml, Aspergillus niger displays zones with widths of 1.940 ± 0.351, 1.7072 ± 0.427, 2.092 ± 0.5623, and 2.3145 ± 0.6616, respectively. Furthermore, the diameter of the zone produced by Staphylococcus aureus and Escherichia coli, respectively, when measured with chloroform extract is 2.31 and 2.661, respectively. Aqueous extract zones for E. coli and Staphylococcus aureus have diameters of 0.980 and 1.432, respectively. When it comes to combating bacteria like Staphylococcus aureus (zone of diameter 2.31), Escherichia coli (zone of 2.661), and Candida albicans (zone of 4.25514 ± 0.814), the aqueous extract of Couroupita guianensis has greater efficacy than the chloroform extract. The following is Aspergillus niger.

The growth of the investigated species in the bacteria and fungi utilized in this experiment was suppressed in diverse ways by aqueous extracts and chloroform. The findings indicate that the extract from shade-dried Couroupita guianensis possesses antifungal and antibacterial qualities against harmful organisms for humans. Preliminary phytochemical study revealed that the chloroform extract contained flavonoids, glycosides, alkaloids, steroids, and triterpenoids, while the aqueous extract's active phytochemical contents were tannins, glycosides, and alkaloids.

Conclusion

The work's findings demonstrated that the plants included bioactive substances, which are linked to the antibacterial qualities of plants.It has previously been shown that extracts from Couroupita guianensis exhibit a broad range of activities. This study assessed the antifungal properties of both the couroupita guianensis aqueous extract and the intrinsic antifungal activity of chloroform. The observed results indicate that while chloroform has antifungal properties on its own, couroupita guianensis chloroform extract exhibits synergistic properties. Couroupita guianensis can be added to drugs for topical antifungal therapy because it is readily available and well tolerated. But more research needs to be done on its cost-effectiveness, safety, and integration into oral formulations.

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