ejpmr, 2016,3(11), 505-516

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTIMICROBIAL ACTIVITY OF THE AQUEOUS AND CHLOROFORM EXTRACTS OF LEAVES OF *COUROUPITA GUIANENSIS* BY WELL DIFFUSION METHOD

Praveen Kumar Uppala[1*], Dr. K. Atchuta Kumar[2], D. J. Vinay Ramji[3] and Uma Shankar Gorla[4]

[1]Assistant Professor, Bhaskara Institute of Pharmacy, Affiliated to Andhra University, Vizianagaram.
[2]Principal & Professor, Bhaskara Institute of Pharmacy, Affiliated to Andhra University, Vizianagaram.
[3]Assistant Professor, Bhaskara Institute of Pharmacy, Affiliated to Andhra University, Vizianagaram.
[4]Assistant Professor, Viswanatha Institute of Pharmaceutical Sciences, Affiliated to JNTU, kakinada, Visakhapatnam.

Corresponding Author: Praveen Kumar Uppala

Assistant Professor, Bhaskara Institute of Pharmacy, Affiliated to Andhra University, Vizianagaram.

Article Received on 12/09/2016 **Article Revised on 01/10/2016** **Article Accepted on 23/10/2016**

ABSTRACT

Objective: To investigate Antimicrobial activity (Antibacterial, Antifungal) of the Aqueous and Chloroform Extracts of Leaves of *Couroupita guianensis* by Well Diffusion Method. **Methods:** The Antimicrobial activity (Antibacterial, Antifungal) of the Aqueous and Chloroform Extracts of Leaves of *Couroupita guianensis* by Well Diffusion Method and the results were compared for the both extracts. Antibacterial activity is compared with standard antibiotic Penicillin (10mg/ml) and antifungal activity is compared with standard antibiotic Clotrimazole (10mg/ml). **Results:** The chloroform extract of *Couroupita guianensis* showed better activity against the fungus like *Candida albicans* with the zone of 4.36±0.84 followed by *Aspergillus niger* with zone of diameter 2.3127±0.668 and the aqueous extract shows better activity against the bacteria like, *Staphylococcus aureus* zone of diameter is 2.2 *Escherichia coli* the zone of diameter 2.67. In the present study, both in bacteria and fungi chloroform and aqueous extracts showed a varying degree of inhibition of the growth against tested organism. **Conclusion:** From the above finding concluding that, this study evaluated the inherent antifungal activity of chloroform as well as the antifungal activity of aqueous extract of *couroupita guianensis*. From the obtained results it can be concluded that although chloroform itself has antifungal activity, chloroform extract of *couroupita guianensis* has a synergistic activity.

KEYWORDS: *Couroupita guianensis, Candida albicans, Aspergillus niger, Staphylococcus aureus, Escherichia coli.*

INTRODUCTION

Plants have been used for medicinal purposes for as long as history has been recorded. Despite the progress in orthodox medicine, interest in alternative medicine, including herbalism, is on the increase in the West and for 80% of the world herbal medicine is still the only kind to which ordinary persons have ready access. A great variety of plants are used for medicinal treatments. Either the dried plant, or a specific part of it (root, leaves, fruit, flowers, seeds), is formulated into suitable preparations — compressed as tablets or made into pills, used to make infusions (teas), extracts, tinctures, etc., or mixed with excipients to make lotions, ointments, creams, etc.

Similarly, many consider that since plants are natural materials they are safer and will produce fewer side-effects than synthetic drugs. There is little substance or reason in either of these claims. For example, comfrey (Symphytum officinale) is considered a safe herb and is used as a demulcent. However, it contains pyrrolizidine alkaloids, which are toxic to the liver and can cause liver cancer. Herbal medicine (or "herbalism") is the study and use of medicinal properties of plants. Studies show that in tropical climates where pathogens are the most abundant, recipes are the most highly spiced. Further, the spices with the most potent antimicrobial activity tend to be selected. In all cultures vegetables are spiced less than meat, presumably because they are more resistant to spoilage.

Among the more popular remedies used are ginseng, to increase stamina and as a mild sedative; St.-John's-wort, for mild depression; echinacea, to aid the immune system and alleviate colds; kava, to calm anxiety and treat insomnia; saw palmetto, for enlarged prostate; and ginkgo biloba, to improve short-term memory.

This widespread use has prompted demands that herbal remedies be regulated as drugs to insure quality

standards. The U.S. Food and Drug Administration (FDA) can require a clinical trial on any herb that has a health claim on its label, but medical testing, which is geared toward observing a particular active component, is difficult to apply to herbs, which may have many interacting ingredients.

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care.

Couroupita guianensis is a deciduous tree belonging to the family Lecythidaceae. It is native to South India and Malaysia. Various part of the tree have been reported to contain oils, keto steroids, glycosides, couroupitine, indirubin, isatin and phenolic substances The pulp of the fruits oxidizes bluish-green when exposed to air and is extremely malodorous probably because of sulphur compounds in the fruits. The fruit contains small seeds in a white, unpleasant smelling edible jelly.The large fruit, which is woody and very spherical, measuring up to 25 centimeters wide, gives the species the common name "cannonball tree". A smaller fruit contains perhaps 65 seeds, while a large one can have 550. One tree can bear 150 fruits. The fruit takes up to a year to mature in most areas, sometimes as long as 18 months. This plant is used for treating mange and other skin conditions. The pulp of the fruit of the cannon ball tree is rubbed on the infected skin of mange dog. It is claimed that when the dog licks its skin, this medicine will also work internally. The flowers are used to cure cold, intestinal gas formation and stomachache.

PLANT PROFILE
Scientific Name:
Couroupita guianensis Aubl.

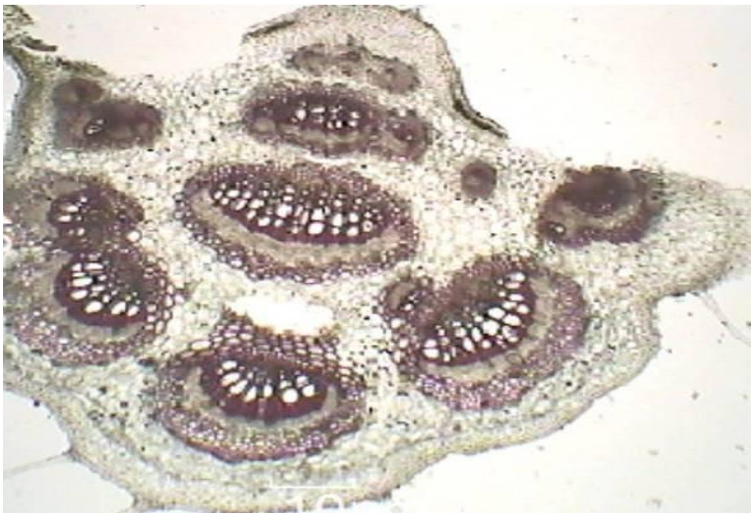
Common Names:
Cannon ball tree, Sal tree, Ayauma tree.

Ethnomedicinal uses

- Leaves of *C.guianensis* are widely used as an analgesics by the Brazilian rural population
- Juice made from the leaves is used to treat skin disorders and the Shamans of South America have even utilized tree parts for curing malaria
- *Couroupita guianensis* infusions or teas obtained from different parts of the tree used traditionally to treat hypertension, tumours, pain and inflammatory processes
- *Couroupita guianensis* flowers are used to cure cold,intestinal gas formation and stomach ache
- In orissa, decoction of flowers has been used to boost the immune system to fight number of diseases
- The fruit pulp of *Couroupita guianensis* is used to disinfect wounds

The plant was collected from Bobbili region, vizianagaram, in the month of December, The leaves were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were shade dried at room temperature for about 10 days. The shade dried leaves were pulverized in mixer grinder to form fine powder and passed through mesh size 100.

Three slides are prepared accordingly with different reagents as following and seen under compound microscope
1. T.S.+Glycerin
2. T.S.+Iodine solution+Glycerin
3. T.S+Phloroglucinol+Con.Hcl+Glycerin



The transverse section of the leaf of *couroupita guianensis* shows the presence of:

- Starch grains
- Anomocytic stomata
- Prism type of calcium oxalate crystals
- Covering trichomes
- Xylem vessels

Preparation of Extracts by successive solvent extraction

The finely powdered leaf drug of *couroupita guianensis* about 80gm was extracted with chloroform(50-55 c) for 72 hours by continuous hot percolation method using soxhlet apparatus.Then it was evaporated to form a dry mass of chloroform extract.

Aqueous extract is prepared by dissolving 500ml distilled water in 500gm of finely powdered leaf drug of *couroupita guianensis* and kept for 36hrs.Then it is filtered and evaporated

ANTIBACTERIAL ACTIVITY

The leaf extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic Penicillin (10mg/ml) in vitro by well diffusion method. Lawn culture was used using the test organism on Nutrient Agar. The inoculated plates were kept aside for few minutes using well cutter, four wells were made in those plates at required distance. In each step of well cutting the well cutter was thoroughly wiped with alcohol. A fixed volume (100µl) of the plant extract was then introduced into the wells in the increasing concentration. The plates with bacteria were incubated at 37˚c for 24 hours. The activity of the extract was determined by measuring the diameters of zone of inhibition.

ANTIFUNGAL ACTIVITY

The leaf extracts were also screened for their antifungal activity in comparison with standard antibiotic Clotrimazole (10mg/ml) in vitro by well diffusion method. Lawn culture was prepared using the test organism on Potato Dextrose Agar (PDA). The inoculated plates were kept aside for few minutes using well cutter, four wells were made in those plates at required distance. A fixed volume (100µl) of the extracts was then introduced into the wells in the increasing concentration. The plates with fungi were incubated at room temperature for 48 hours. The activity of the extract was determined by measuring the diameters of zone of inhibition.

For antibacterial sensitivity nutrient agar/broth (P^H 7.4) and Pototao dextrose agar (P^H 5.5-6) etc. Fresh inocula are prepared for in-vitro sensitivity test. For bacteria,the test in colums are obtained from 24^{th} old culture.The colony forming units of the test inocula is approximately 105 cells/ml for testing.Temperature and period of incubation,antibacterial sensitivity test results are invariably read after 18-24 hrs for incubation at 37^0C.

Four human pathogenic microorganisms, such as *Staphylococcus aureus, Escherichia coli* and Fungi *Aspergillus niger* and *Candida albicans* were used in the study for the evaluation of the antimicrobial activity. Among which Gram positive and gram negative Strains of bacteria and 2 Strains of Fungus were isolated in the laboratory. All the strains were collected from the Department of Microbiology, Bhaskara Institute of Pharmacy,Komatipalli, Bobbili.

TABULATION OF THE NAME OF THE STRAIN AND THEIR TYPE

SL NO	***STRAIN NAME***	***TYPE***
1	*Candida albicans*	Human pathogenic
2	*Aspergillus niger*	Human pathogenic
3	*Streptococcus aureus(G+VE)*	Human pathogenic
4	*E.coli(G-VE)*	Human pathogenic

Nutrient agar for bacteria (PH 7.4)

Composition	**Quantity**
Peptone	5gms
Beef extract	3gms
Nacl	5gms
Agar	20gms
Distilled water	1000ml

Potato dextrose agar for Fungi

Composition	**Quantity**
Dextrose	15.0gms
Peeled Potato	100gms
Agar	15.0gms
Distilled water	1000ml
Ph	5.6

PREPARATION AND STERILISATION OF MEDIUM

Agar (35gm) medium was mixed with one litre of distilled water, enclosed in a screw cap container and autoclaved at 121^0C for 15 min. The medium was later dispensed into 90mm sterile agar plates and left to set.

The agar plates were incubated for 37^0C to confirm their sterility. When no growth occurred after 24 hours, the plates were considered sterile. Standard culture of four human pathogenic microorganisms, such as *Staphylococcus aureus, Escherichia.coli, Aspergillus* niger and *Candida albicans* were chosen for the study.

PLATING THE MEDIA

The antibacterial activity screening was done as described before. Briefly, cultures of the microorganisms from culture plates were scooped using a wire loop and separately mixed using normal saline and agitated with vortex mixer. A loop fill was withdrawn and introduced into the sterilized and cooled Nutrient agar media aseptically by following the Pour plate technique.

Wells of approximately 4mm in diameter and 2.5mm deep were made on the surface of the solid medium using a sterile borer. The plates were turned upside down and the wells labeled with a marker.

After 24 hours the plates were removed and zone of inhibition was measured by zone reader and the results were tabulated.

ANTIBIOTICS USED AS STANDARDS

Organisms	Antibiotic used as Standard
Staphylococcus aureus (G +ve)	Penicillin
E.coli (G -ve)	Doxycycline
Aspergillus niger	Clotrimazole
Candida albicans	Clotrimazole

RESULTS AND DISCUSSION

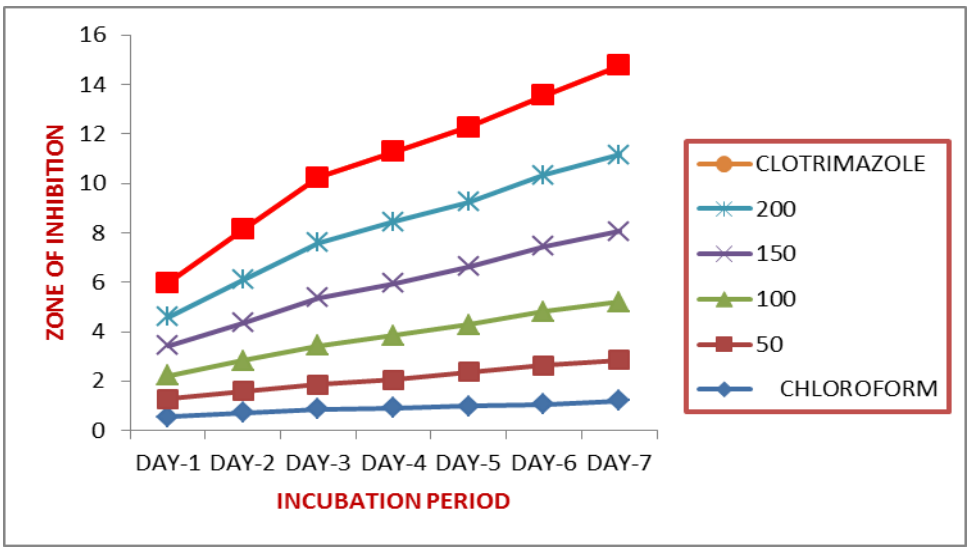
QUALITATIVE ANALYSIS OF BIOACTIVE COMPOUNDS IN DIFFERENT SOLVENT EXTRACTS OF *Couroupita guianensis* LEAVES

Test name	Chloroform extract	Aqueous extract
Mayer's	++	+
Wagner's	++	+
Dragendroff's	++	+
Tannins	+	+
Phlobatannins	++	+
Glycosides	++	++
Sterols	++	+
Resins	++	+++
Phenols	+	+
Anthraquinones	++	+
Carbohydrates	++	++
Cardiac glycosides	-	_
Steroids	+	+
Terpenoids	++	++
Alkaline reagent Test	+	+

INHIBITION ZONE DIAMETER OF DIFFERENT EXTRACTS OF *couroupita guianensis* AGAINST DIFFERENT FUNGUS

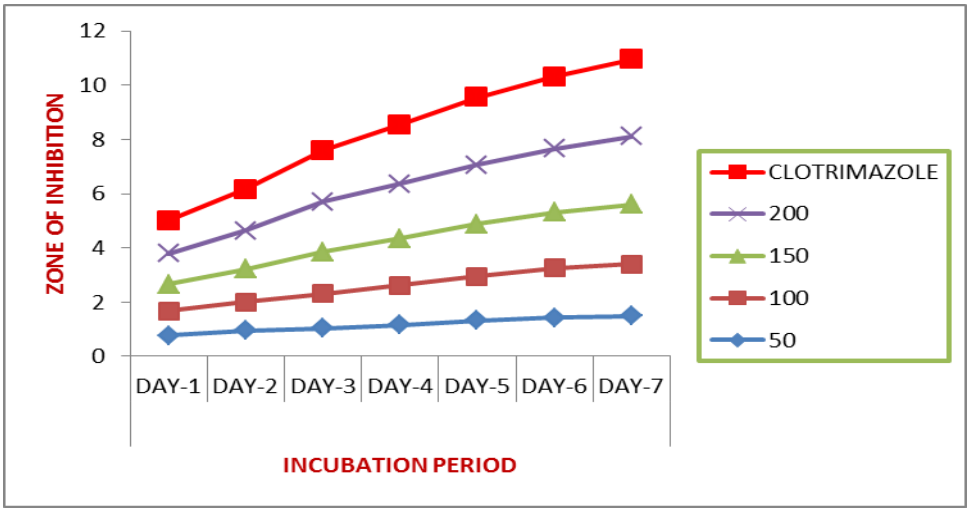
Effect of Chloroform extract on *Aspergillus niger*

Concentrations (mg/ml)	DAY-1	DAY-2	DAY-3	DAY-4	DAY-5	DAY-6	DAY-7
50	0.72	0.89	1	1.15	1.38	1.57	1.65
100	0.93	1.23	1.55	1.78	1.93	2.18	2.35
150	1.21	1.53	1.96	2.11	2.33	2.64	2.85
200	1.18	1.74	2.22	2.48	2.63	2.87	3.1
Chloroform	0.56	0.71	0.87	0.91	0.98	1.07	1.2
CLOTRIMAZOLE (10µg/ml)	1.38	2.02	2.64	2.85	3.01	3.23	3.6



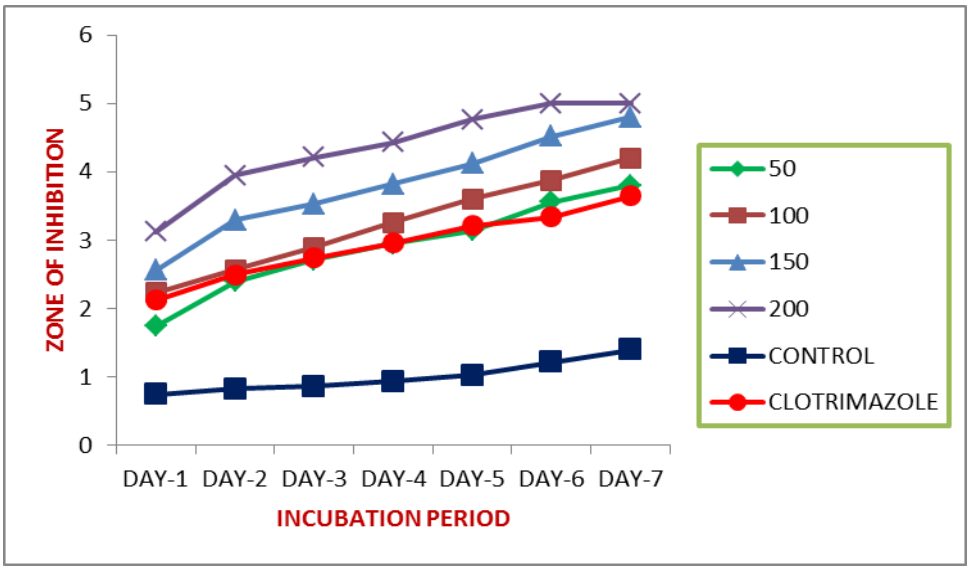
Effect of Aqueous extract on *Aspergillus niger*

Concentrations (mg/ml)	DAY-1	DAY-2	DAY-3	DAY-4	DAY-5	DAY-6	DAY-7
50	0.78	0.95	1.03	1.16	1.32	1.43	1.5
100	0.9	1.05	1.29	1.47	1.63	1.83	1.9
150	0.98	1.21	1.53	1.72	1.93	2.06	2.2
200	1.13	1.42	1.85	2.02	2.18	2.34	2.5
Aqueous	-	-	-	-	-	-	-
CLOTRIMAZOLE (10µg/ml)	1.22	1.53	1.90	2.17	2.49	2.64	2.85



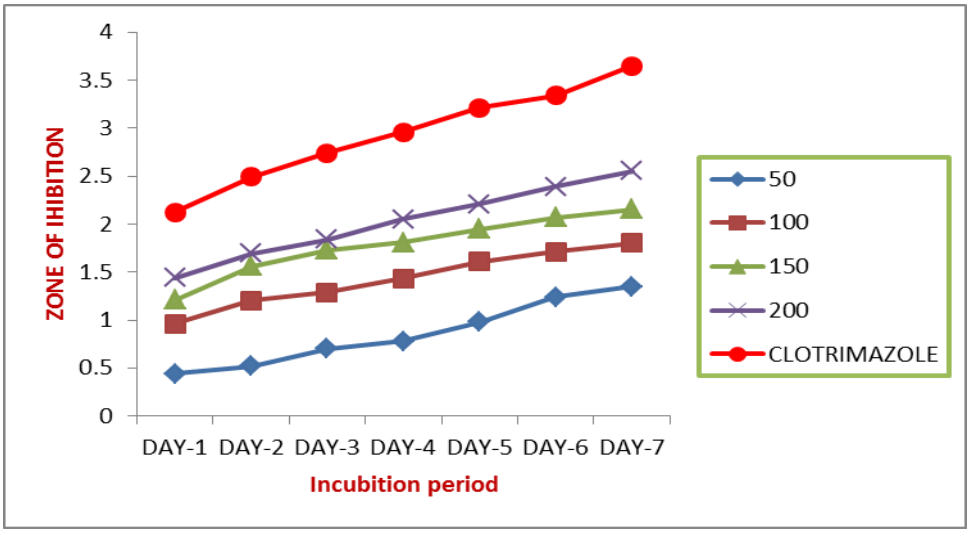
Effect of Chloroform extract on ***Candida albicans***

Concentrations (mg/ml)	DAY-1	DAY-2	DAY-3	DAY-4	DAY-5	DAY-6	DAY-7
50	1.74	2.39	2.71	2.95	3.14	3.56	3.8
100	2.23	2.57	2.89	3.26	3.60	3.87	4.2
150	2.56	3.29	3.53	3.82	4.12	4.52	4.8
200	3.12	3.95	4.21	4.43	4.76	5	5
CONTROL	0.74	0.82	0.86	0.93	1.02	1.21	1.4
CLOTRIMAZOLE µg/ml	2.12	2.49	2.74	2.96	3.21	3.34	3.65



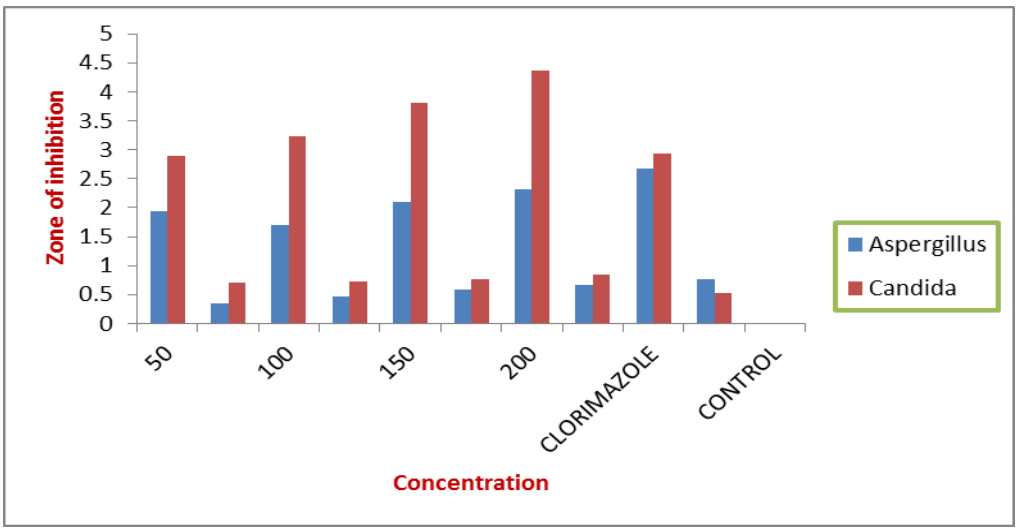
Effect of Aqueous extract on *Candida albicans*

Concentrations (mg/ml)	DAY-1	DAY-2	DAY-3	DAY-4	DAY-5	DAY-6	DAY-7
50	0.44	0.52	0.7	0.78	0.98	1.24	1.35
100	0.96	1.2	1.29	1.43	1.61	1.71	1.8
150	1.21	1.56	1.73	1.81	1.95	2.07	2.15
200	1.44	1.69	1.84	2.05	2.21	2.39	2.55
Aqueous	-	-	-	-	-	-	-
CLOTRIMAZOLE µg/ml	2.12	2.49	2.74	2.96	3.21	3.34	3.65



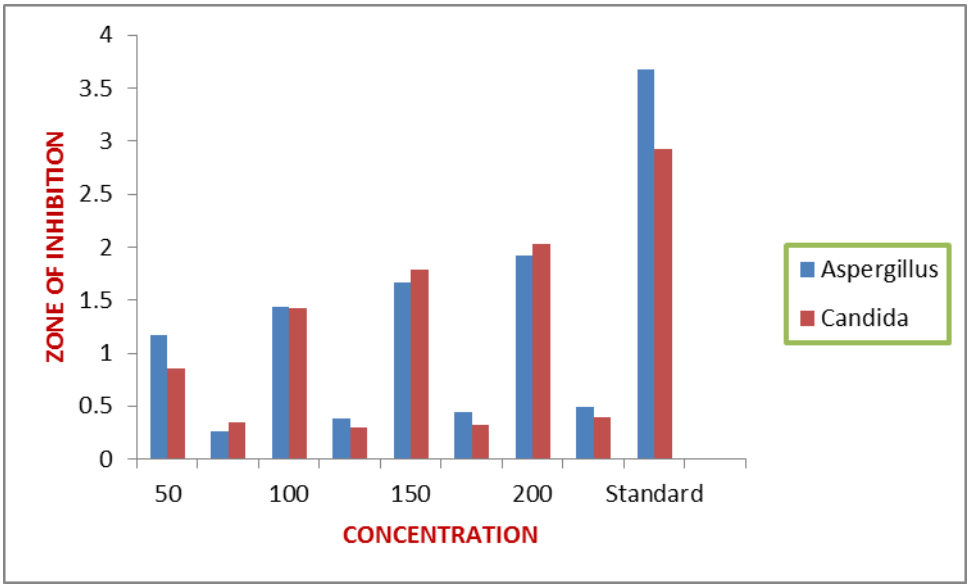
EFFECT OF CHLOROFORM EXTRACT ON ***Aspergillus niger* & *Candida albicans***

Concentration(mg/ml)	*Aspergillus niger*	*Candida albicans*
50	**1.942 ± 0.352**	**2.8985 ± 0.701**
100	**1.7071 ± 0.457**	**3.2314 ± 0.72**
150	**2.09 ± 0.584**	**3.8057 ± 0.77**
200	**2.3127 ± 0.668**	**4.36 ± 0.84**
Chloroform	**0.9 ± 0.2152**	**0.9971 ± 0.2341**
Clotrimazole	**2.67 ± 0.76**	**2.93 ± 0.2341**



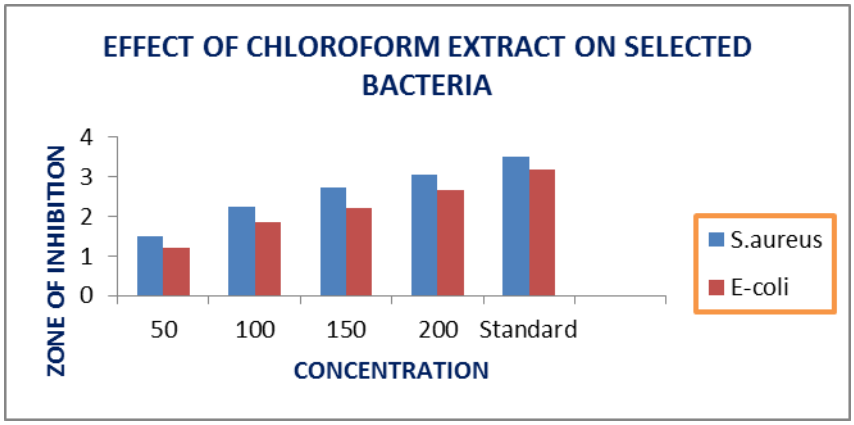
EFFECT OF AQUEOUS EXTRACT ON *Aspergillus niger* & *Candida albicans*

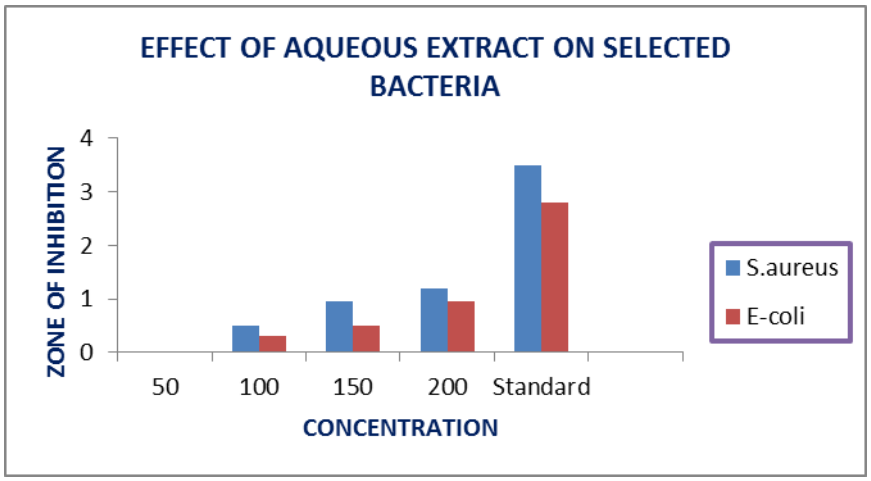
Concentration(mg/ml)	*Aspergillus niger*	*Candida albicans*
50	1.1671 ± 0.2643	0.85857 ± 0.3471
100	1.4385 ± 0.3801	1.4285 ± 0.3003
150	1.6614 ± 0.449	1.7828 ± 0.3231
200	1.92 ± 04948	2.024 ± 0.3942
Water	-	-
Clotrimazole	3.67 ± 0.65	2.93 ± 0.5252

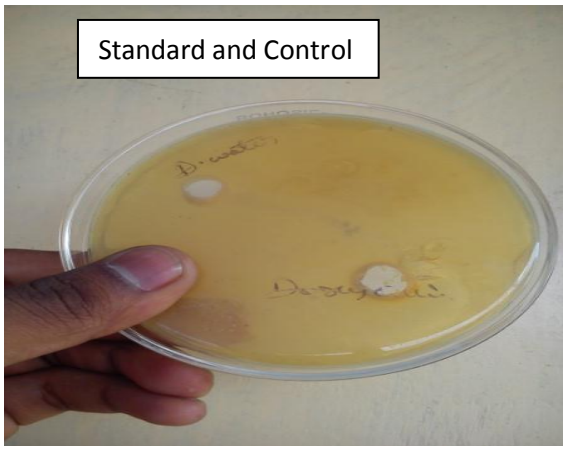


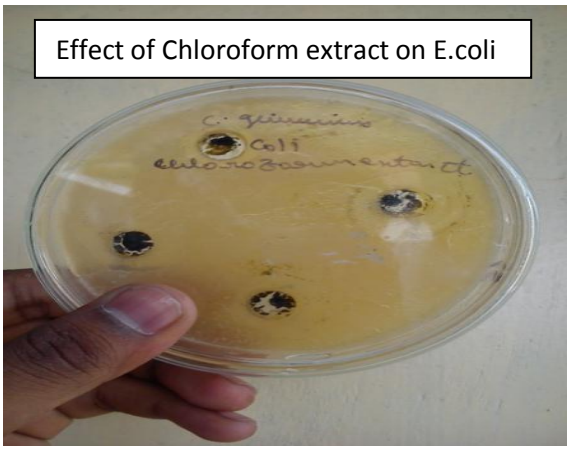
EFFECT OF DIFFERENT EXTRACTS OF *Couroupita guianensis* AGAINST SELECTED BACTERIA

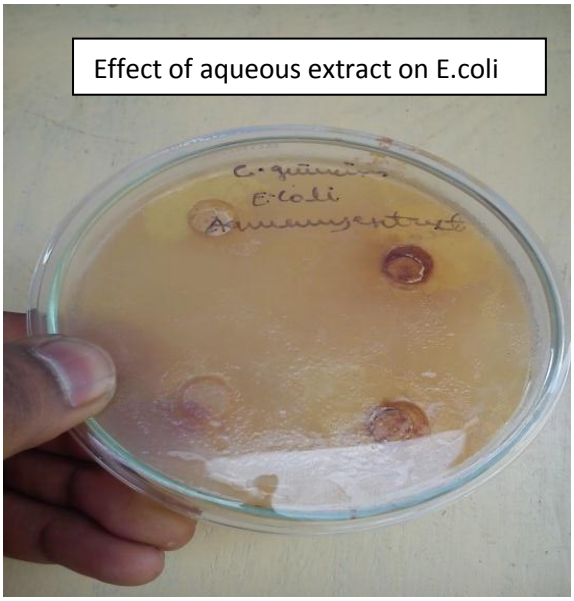
Organism				Organism			
Staphylococcus aureus				E.Coli			
CHLOROFORM		AQUEOUS		CHLOROFORM		AQUEOUS	
Concentration (mg/ml)	Zone of Inhibiton(cm)	Concentration (mg/ml)	Zone of Inhibition(cm)	Concentration (mg/ml)	Zone of Inhibition(cm)	Concentration (mg/ml)	Zone of Inhibition(cm)
50	0.5	**50**	-	**50**	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
Penicillin 10µg/ml	3.5	**Penicillin 10µg/ml**	3.5	**Doxycylin 10µg/ml**	2.8	**Doxycylin 10µg/ml**	2.8

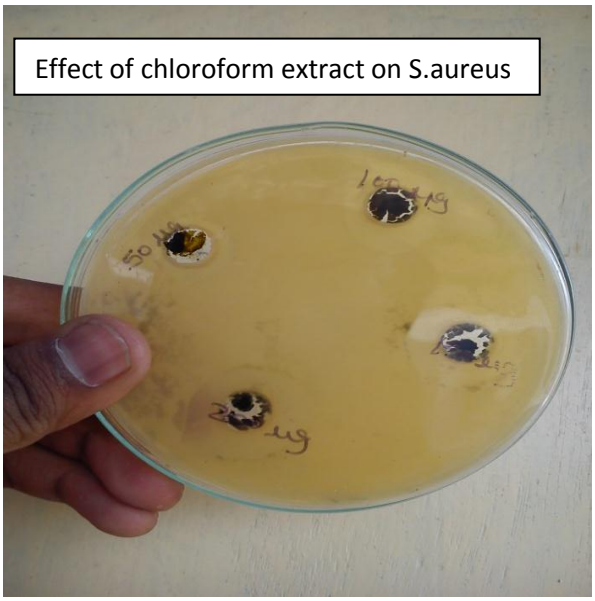

EFFECT OF CHLOROFORM EXTRACT ON SELECTED BACTERIA
ZONE OF INHIBITION
4
3
2
1
0
50
100
150
200
Standard
CONCENTRATION
S.aureus
E-coli

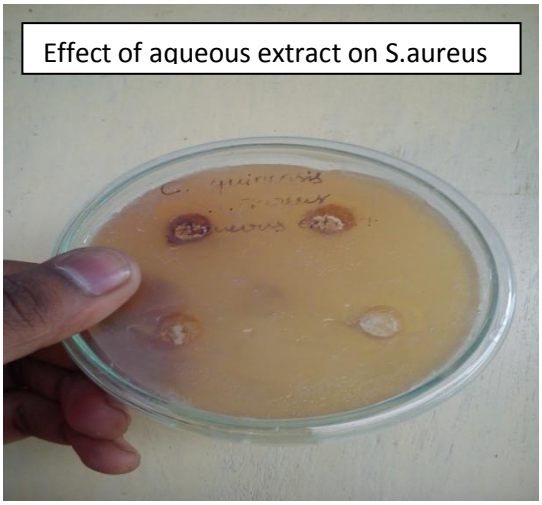

EFFECT OF AQUEOUS EXTRACT ON SELECTED BACTERIA
ZONE OF INHIBITION
4
3
2
1
0
50
100
150
200
Standard
CONCENTRATION
S.aureus
E-coli


Standard and Control


Effect of Chloroform extract on E.coli







DISCUSSION

The leaf extracts of ***Couroupita guianensis*** were screened for its antimicrobial and pharmocological activities. The solvents used for the leaves extraction were chloroform and water. The extract was tested against infectious diseases causing fungal *pathogens* such as ***Aspergillus niger***, ***Candida albicans*** and Bacterial pathogens such as ***Staphylococcus aureus*** and ***Escherichia coli*** using the Agar well diffusion method. The chloroform extract of ***Couroupita guianensis*** showed more activity against fungus like *Candida albicans* and the zone of diameter 2.8985 ±0.701, 3.2314±0.72, 3.8057±0.77 & 4.36±0.84 for the concentrations 50,100,150,200 mg/ml respectively whereas on *Aspergillus niger*, the zone of diameters are 1.942±0.352, 1.7071±0.457, 2.09±0.584 & 2.3127±0.668 for the concentrations 50,100,150,200mg/ml respectively and bacteria like *Staphylococcus aureus*, the zone of diameter is 2.2 by chloroform extract, *Escherichia coli* zone of diameter 2.67 by chloroform extract, and on *Staphylococcus aureus* zone of diameter is 1.2 by aqueous extract and on E.coli zone of diameter is 0.96

The chloroform extract of *Couroupita guianensis* showed better activity against the fungus like *Candida albicans* with the zone of 4.36±0.84 followed by *Aspergillus niger* with zone of diameter 2.3127±0.668 and the aqueous extract shows better activity against the bacteria like, *Staphylococcus aureus* zone of diameter is 2.2 *Escherichia coli* the zone of diameter 2.67. In the present study, both in bacteria and fungi chloroform and aqueous extracts showed a varying degree of inhibition of the growth against tested organism. The results confirmed that presence of Antifungal and Antibacterial activity in the shade dried extract of *Couroupita guianensis* against the human pathogenic organisms. 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.

CONCLUSION

The antimicrobial activity of the different extracts of this cannon ball tree was assessed against various human

pathogenic bacteria. Plant based antimicrobial have enormous therapeutic potential as they can serve the human with lesser side effects and boon for the development of chemotherapy. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activities.

The present study was carried out with a vision to setup standards that could be beneficial for detecting the authenticity of this vital medicinal plant.Numerical standards reported in this work could be useful for the compilation of a suitable monograph of *Couroupita guianensis*. As the plant produce secondary metabolites in order to protect themselves from microorganism, herbivores and insects, thus antimicrobial effect is somehow expected from plants namely flavonoids, alkaloids, tannins, saponins and glycosides are producing a better opportunity for testing wide range of microorganism. The results obtained from this work revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants.

The wide spectrum of activity of Couroupita guianensis extracts has been documented earlier. This study evaluated the inherent antifungal activity of chloroform as well as the antifungal activity of aqueous extract of couroupita guianensis. From the obtained results it can be concluded that although chloroform in itself has antifungal activity, chloroform extract of couroupita guianensis has a synergistic activity. Since couroupita guianensis is easily available and well tolerated, it can be incorporated into medications for topical antifungal therapy. However, further studies for its incorporation into oral preparations, safety and cost- effectiveness has to be conducted.

REFERENCES

1. V. E. Tyler and S. Foster, Tyler's Honest Herbal (rev. ed. 1999); The Physicians' Desk Reference for Herbal Medicines (annual).
2. Lichterman, B. L (2004). "Aspirin: The Story of a Wonder Drug". British Medical Journal 329 (7479): 1408. doi:10.1136/bmj.329.7479.1408.
3. Tapsell LC, Hemphill I, Cobiac L, et al. (August 2006). "Health benefits of herbs and spices: the past, the present, the future". Med. J. Aust. 185 (4 Suppl): S4–24. PMID 17022438.
4. Lai PK, Roy J (June 2004). "Antimicrobial and chemopreventive properties of herbs and spices". Curr. Med. Chem. 11 (11): 1451–60. PMID 15180577.
5. Fabricant DS, Farnsworth NR (March 2001). "The value of plants used in traditional medicine for drug discovery". Environ. Health Perspect. 109 Suppl 1 (Suppl 1): 69–75. PMC 1240543. PMID 11250806.
6. P. E. Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India. 411 044 and Jawaharlal Nehru Technological University, JNTU), Hyderabad, Andhra Pradesh, India 500072.
7. Kavitha R, Kamalakannan P, Deepa T, Elamathi R, Sridhar S*Suresh Kumar J." Invitro Antimicrobial Activity and Phytochemical Analysis of Indian Medicinal Plant Couroupita guianensis Aubl". J. Chem. Pharm. Res., 2011; 3(6): 115-121.
8. A. Elumalai*, V. Naresh, M. Chinna Eswaraiah, P. Narendar, Raj Kumar." Evaluation of Antiulcer Activity of Couroupita guianensis Aubl Leaves". 2012; Vol. 2: Issue 2, Pg 64.
9. Mariana M.G. Pinheiro, Sidnei B.O. Fernandes, Catarina E. Fingolo , Fábio Boylan, Patrícia D. Fernandes ." Anti - inflammatory activity of ethanol extract and fractions from *Couroupita guianensis* Aublet leaves."Journal of Ethnopharmacology. Vol. 146: Issue 1,7 march 2013.
10. Sanjay.Prahalad Umachigi*, Jayaveera K.N., Ashok kumar C.K., G.S. Kumar." Antimicrobial, Wound Healing and Antioxidant potential of Couroupita guianensis in rats ". (2007).page no: 269.
11. Patrícia Dias Fernandes, Mariana M.G. Pinheiro , Sidnei B.O. Fernandes , Catarina E. Fingolo , Fábio Boylan." Antinociceptive activity of fractions from *Couroupita guianensis* Aubl. Leaves."Journal of Ethnopharmacology.Vol.127, Issue 2,3 february 2010.
12. Mariappan Premanathan, Srinivasan Radhakrishnan, and Kandasamy Kathiresan. "Antioxidant & anticancer activities of isatin (1H-indole-2,3-dione), isolated from the flowers of *Couroupita guianensis* Aubl". Indian J Med Res.2012 November; 136(5): 822-826.
13. MEDICINAL PLANTS, P. P. JoyJ. ThomasSamuel MathewBaby P. Skaria, Kerala Agricultural University, Aromatic and Medicinal Plants Research Station,Ernakulam, Kerala, 1998.
14. Heywood V H, Chant S R. Popular Encyclopedia ofPlants. Cambridge University press, Cambridge, 1982; 103.
15. Lewis Y S. Flowers of Couroupita guianensis Aubl.Current Science 1964; 33: 682.
16. Ahire A. E., Laddha K.S., Beta amyrin palmitate–isolation from Couroupita guianensis Aubl. Leaves.Indian Drugs 2002; 39: 216-216.
17. Jan Bergman, Jan-ol of Lindstrom, Ulf Tilstam. 1985. Thestructure and properties of some indolic constituents inCouroupita guianensis Aubl. Tetrahedron 41: 2879-2881.
18. Wong M, Licinio J. Research and treatment approaches to depression. Nat Rev Neurosci 2001; 2: 343–51.
19. Nestler EJ, Barrot M, Di Leonem RJ, Eisch AJ, Gold SJ,Monteggia L. M. Neurobiology of depression. Neuron 2002; 34: 13–25.