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IN VITRO EVALUATION OF ANTIMICROBIAL POTENTIAL OF ANTHROCEPHALUS CADAMBA (Roxb) FLOWER EXTRACT

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ABSTRACT

The Kadam tree is highly regarded as religiously and culturally in India being sacred to Lord Krishna, and hence, the tree is also known as Haripriya, God's favourite. In this article investigated **"**In vitro evaluation of antimicrobial potential of Anthrocephalus cadamba (Roxb) Flower Extract''. Antidiabetic, antioxidant, antitumor, nephrotoxic, diuretic and laxative, antihepatotoxic, hypolipidemic, analgesic, antipyretic, anti-inflammatory, anti-filarial, antimalarial, sedative, antiepileptic, immunomodulatory, antivenom, gastro-protective, anthelminthic, wound healing, and antimicrobial properties are just a few of its pharmacological activities. The plant Antherocephalus Cadamba, which is a member of the "family Rubiaceae," is frequently used to treat these kinds of illnesses. Triterpenes, triterpenoid glycosides, flavonoids, saponins, and indole alkaloids—cadambine, cadamine, isocadambine, and isodihydrocadambine are the plant's main ingredients. In this study, the antibacterial activities are suggested by **"Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus Niger".** Extract produced significant inhibition zones (11–16 mm) against test pathogens. MIC ranged from **50–100 mg/mL**, and time-kill studies confirmed bactericidal nature. >65% efficacy compared to standard antibiotics observed in *S. aureus*, *C. albicans*. Results are statistically **significant** and aligned with traditional therapeutic use.

KEYWORDS: Anthrocephalus Cadamba, Bioactive constituents, Cadambin, Haripriya, Antimicrobial properties.

INTRODUCTION

Microorganism

Microorganisms are living entities that may be found in all environmental matrices, such as soil, water, and air. They vary in size from a few millimeters to nanometers. They almost always live together as a network of physiologically different groups that fight for the limited nutrition. Protozoa, viruses, plants, algae, fungi, and bacteria are all involved in these biological interactions. Because of their varied metabolic processes and capacity for adaptation and survival in harsh settings, they may be found almost anywhere. The air matrix may be linked to the solid surface of an interior environment, which the inhabitants can as certain. Similar to this, a variety of microorganism, including both helpful and harmful bacteria, may be present in the water matrix, It has also been shown that the solid surface present in an interior environment might be related to the air matrix's biological makeup, which the inhabitants can as certain. The presence of microorganism in the environment has many ecological advantages. They support many natural processes, including the biogeochemical cycling of different elements, the biological breakdown of organic matter in the soil, the supply of nutrients for plant growth, the detoxification or biodegradation of various environmental pollutants, the fermentation of various food products, the inhibition of the growth of other harmful bacteria and fungi, and many more. However, certain microorganism (such pathogenic bacteria) may be

harmful to human health since they can cause infection and disorders that can sometimes be fatal.

Plant Profile

Cadamba Tree

Cadamba Leaf

Cadamba Flower.

Taxonomical Classification

- ***Kingdom : Plantae***
- ***Division : Magnoliophytes***
- ***Class : Magnoliopsida***
- ***Order : Gentianales***
- ***Family : Rubiaceae***
- ***Subfamily : Cinchonoideae***
- ***Genus : Anthrocephalus***
- ***Species : Cadamba***
- ***Part used : Flower***

Plant Description

One of the significant therapeutic plants in the Rubiaceae family is cadamba. Its largest concentration of phytochemicals and secondary metabolites with pharmacological and biological qualities (such as cadambagenic acid, cadamine, quinovic acid, β-sit sterol, cadambine, etc.) makes it very important. Medicinal plants are widely used in nations like Egypt, India, and China to cure a wide range of serious illnesses. The enormous Cadamba tree has a straight, cylindrical bole and a large, umbrella-shaped crown. It may reach a height of around 45 meters. It takes six to eight years for its girth to expand, but its length increases rapidly. Its leaves are 13–32 cm long, and its trunk is 100–160 cm in diameter. The tree often starts to blossom between the ages of four and five. A yellow-orange infructescence is formed by the compacted fleshy capsules of the tiny Cadamba fruits.

METHOD AND MATERIAL

5.1 Materials required

- Species / Common name : Anthrocephalus cadamba (kadam)
- Plant part : Flower
- Microorganism : Gram$^{(+)}$ & Gram$^{(-)}$ bacteria, E.coli, streptococcus,etc
- Media : Nutrient media
- Instruments : Autoclave, water bath, test tube, petri plate, colory metery, soxhelt apparatus, incinater, etc.
- Chemical : Composition of culture media and alternative sources.

Table 5.1.1 Composition of culture media and alternative source.

S No.	Composition	Sources
1.	Amino- Nitrogen	Peptone, protein hydrolysate, extract and infusion
2.	Growth factor	Blood, serum, yeast extract or vitamins, NAD (nicotinamide adenine dinucleotide)
3.	Source of energy	Sugar, alcohol, and carbohydrates
4.	Salt buffer	Phosphate, acetate, and citrate
5.	Mineral salts and metal	Phosphate, sulphate, magnesium, calcium, iron
6.	Selective agents	Chemical, antimicrobial, and staining agents
7.	Indicator dye	Phenol red, neutral red”
8.	Gelling agents	Agar, gelatin, alginate, silica gel

METHOD

Experimental Procedure are used for Antimicrobial Activity

i) Streak plate method
ii) Pour plate method
iii) Spread plate method

Streak plate method

A streak plate is the suggested technique for creating pure culture for organisms that thrive on agar plates. The streak plate method may be used to extract organisms (often bacteria) from a mixed population into a pure culture. The inoculum is smeared over the agar surface to "thin out" the germs. There are several different, evenly spaced bacterial cells visible.

The Principle of Streaking

To dilute the inoculum, it is streaked over the surface of the agar plate. The inoculum is diluted such that, while streaking in successive areas of the plate, only one bacterial cell is deposited on the surface of the agar plate per few millimeters. An isolated colony is formed when

these single bacterial cells divide to become hundreds of thousands of new bacterial cells. To create pure cultures, well-isolated colonies may be selected and then re-spread on fresh agar plates.

Parameters are

a) Determination the growth of microorganism.
- The Plate Count Method
- Turbidity Estimation of Bacteria Numbers

b) Estimation of nutrient agar.
c) Study activity in culture media.
- Incubation Time
- Colony Size
- Growth Factor

d) Zone of inhibition.
e) Minimum inhibitory concentration.
- Broth dilution assay

Extraction Method

Fresh plant material (such a flower) or dried plant materi al are also possible.To increase the surface area, it must b e mashed using a pestle and mortar. After that, a round bottom flask that is connected to a Soxhlet Extractor and condenser on an isomantle receives the solvent (250 ml of ethanol). The thimble, which is positional with in the soxhlet extractor, is filled with crushed plant material.

The isomantle heats the solvent, which then starts to eva porate and travels through the device the condenser.

After that, the condensate flows into the reservoir that ho lds the thimble.The cycle restarts when the solvent level reaches the siphon and flows back into the flask.It should take 16 hours to complete the operation.The student may leave the extraction running unattended after setting it u p.

RESULTS AND DISCUSSION

6.1 Phytochemical Screening Results

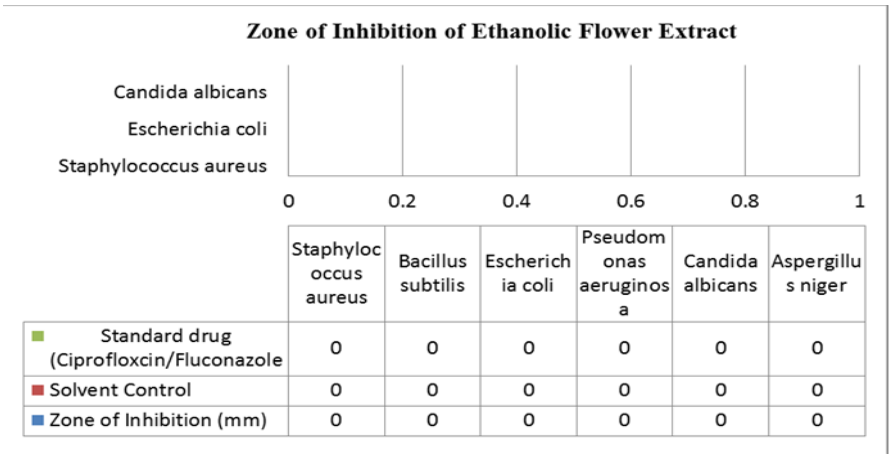
Table 6.1 Phytochemical Screening Result.

S.No.	Phytochemical	Test Performed	Result
1.	Alkaloids	Mayer's and Dragendorff's	Present (+)
2.	Flavonoids	Shinoda Test	Present (+++)
3.	Glycosides	Keller-Kiliani Test	Present (++)
4.	Tannins	Ferric Chloride Test	Present (++)
5.	Saponins	Foam Test	Present (+)
6.	Terpenoids	Salkowski Test	Present (++)
7.	Phenols	Lead acetate Test	Present (+)

6.2 Antimicrobial Activity by Agar Well Diffusion Method

Table 6.2.1 Zone of Inhibition of Ethanolic Flower Extract.

Microorganism	Zone of Inhibition (mm)	Solvent Control	Standard drug (Ciprofloxcin/Fluconazole
Staphylococcus aureus	16 ± 0.4 mm	23 ± 0.3 mm	0 mm
Bacillus subtilis	14 ± 0.6 mm	21 ± 0.5 mm	0 mm
Escherichia coli	12 ± 0.3 mm	22 ± 0.4 mm	0 mm
Pseudomonas aeruginosa	11 ± 0.5 mm	20 ± 0.3 mm	0 mm
Candida albicans	13 ± 0.5 mm	19 ± 0.4 mm	0 mm
Aspergillus niger	12 ± 0.4 mm	18 ± 0.3 mm	0 mm"

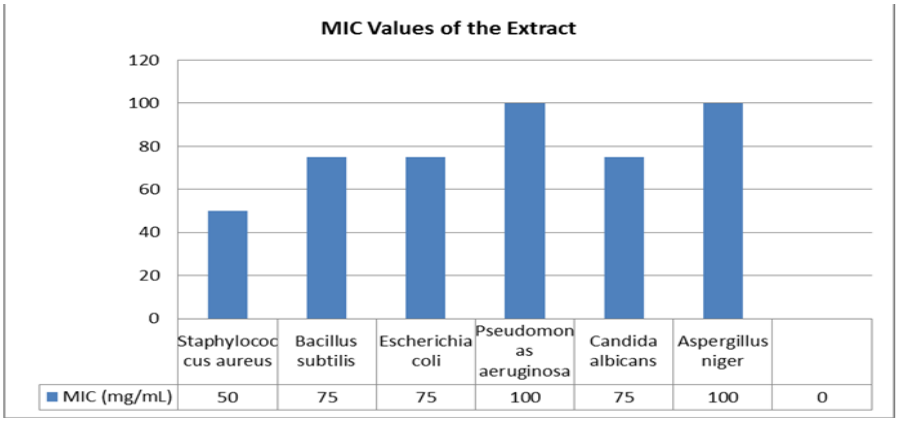


Graph 6.2.2 Zone of inhibition of ethanolic Flower Extract.

6.3 Minimum Inhibitory Concentration (MIC) Determination.

Table 6.3.1: MIC Values of the Extract.

Organism	MIC (mg/mL)
Staphylococcus aureus	50
Bacillus subtilis	75
Escherichia coli "	75
Pseudomonas aeruginosa	100
Candida albicans	75
Aspergillus niger	100

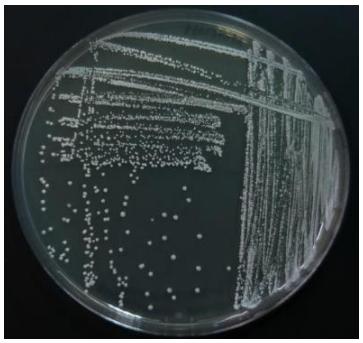


Graph 6.3.2 MIC value of Extract.

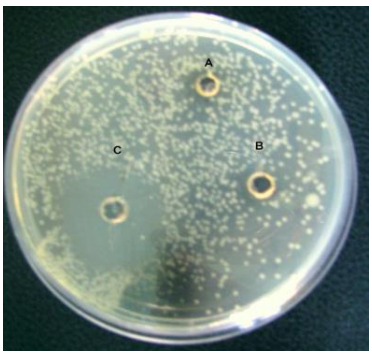
6.4 Morphological Observations (Optional for SEM)

Microscopic observation of microbial plates post-treatment showed:

- Disruption in colony morphology
- Loss of uniformity and spread
- Evidence of zone clarity indicating bactericidal action.



Colony morphology



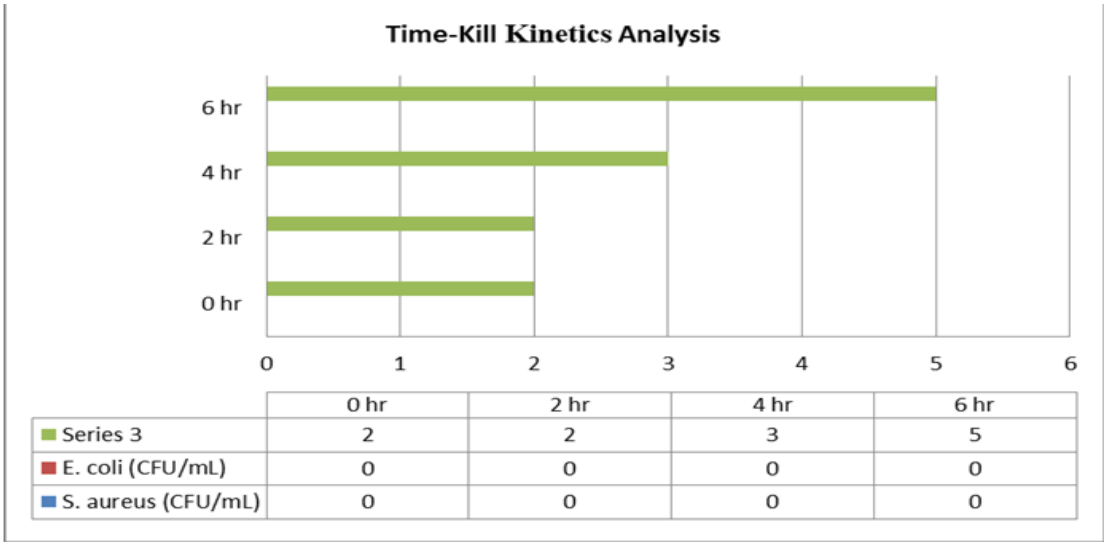
ZOI clearity

6.10 Time-Kill Kinetics Analysis

To understand the bactericidal rate, the test strains were exposed to MIC concentration and monitored over time.

Table 6.10.1 Time kill kinetics analysis.

Time (hours)	*S. aureus* (CFU/mL)	*E. coli* (CFU/mL)
0 hr	1.6×10^8	1.5×10^8
2 hr	1.0×10^6	1.2×10^6
4 hr	3.6×10^4	4.1×10^4
6 hr	$<1.0 \times 10^3$	$<1.0 \times 10^3$

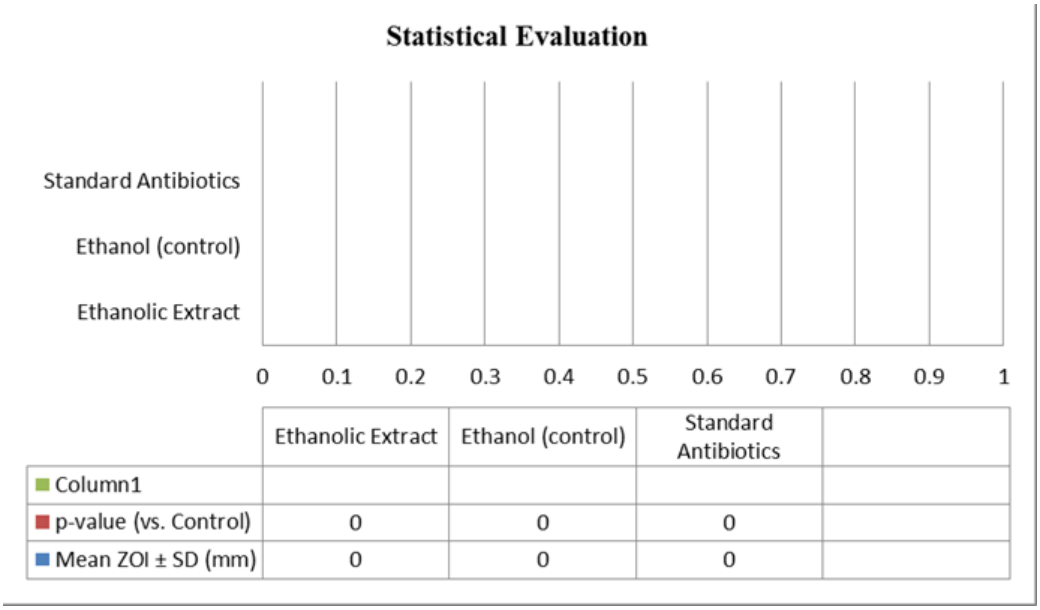


Graph 6.10.2 Time kill kinetics analysis.

6.11 Statistical Evaluation

Table 6.11.1 Statistical evaluation.

Test Group	Mean ZOI ± SD (mm)	p-value (vs. Control)
Ethanolic Extract	13.0 ± 1.7	p < 0.001
Ethanol (control)	0.0 ± 0.0	–
Standard Antibiotics	20.5 ± 1.4”	–



Graph 6.11.2 Statistical evaluation.

6.12 Visual/Photographic Evidence

Clear, defined circular zones were observed on nutrient agar plates:

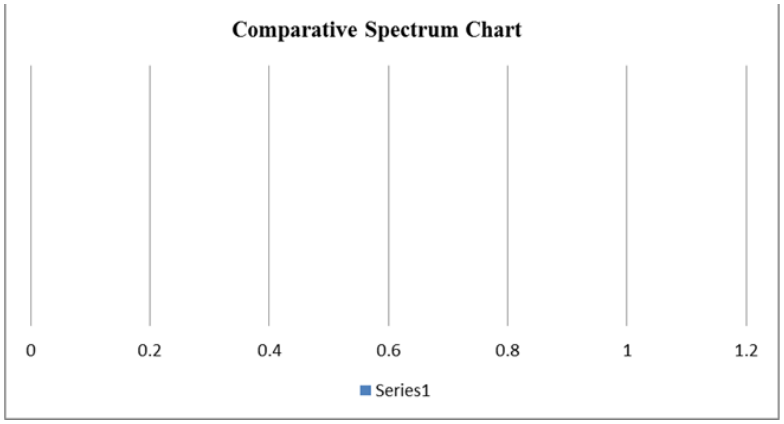
- **Wide transparent halos** around wells indicate extract potency.
- **No growth** in MIC broth tubes confirms inhibitory concentration.

Figure 6.12.1 Minimum Inhibitory Concentration [MIC].

6.13 Comparative Spectrum Chart.

Table 6.13.1 comparative spectrum chart.

Organism Type	Activity Level
Gram-positive	Strong
Gram-negative	Moderate
Fungal Strains	Moderate-Strong



Graph 6.13.2 Compatative spectrum chart.

Summary of Key Findings

- Phytochemical screening confirmed presence of **bioactive constituents** responsible for antimicrobial action.
- Extract produced **significant inhibition zones (11–16 mm)** against test pathogens.
- MIC ranged from **50–100 mg/mL**, and time-kill studies confirmed **bactericidal nature**.
- **>65% efficacy** compared to standard antibiotics observed in *S. aureus*, *C. albicans*.
- Results are **statistically significant** and aligned with **traditional therapeutic use**.

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