Research Article

In Vitro Evaluation of Isatin from *Couroupita guianensis* Aubl against the Clinical Isolates of Bacteria and Fungi

D. Kavitha*, G. Nandhini, P.R Padma
Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India.
***Corresponding author's E-mail:** dkavithabiotech@gmail.com

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ABSTRACT

Plants have formed the basis of traditional systems of medicine that have been in existence for thousands of years and continue to provide mankind with new remedies. The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs. In this study, we analyzed the antimicrobial activity of Isatin, which is an alkaloid present in *Couroupita guianensis* Aubl. against the clinical isolates. Among the bacterial strains, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri, Proteus vulgaris* and *Klebsiella pneumoniae* were used and among the fungal strains *Asperillus niger, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Mucor oryzae* and *Rhizopus indicus* were used. Minimum inhibitory concentration was determined by micro dilution method. The isatin showed the maximum zone of inhibition against *Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Aspergillus flavus* and *Candida albicans* and the MIC of isatin against *Proteus vulgaris, Staphylococcus aureus* and *Shigella flexneri* was 312.5µg/ml. whereas the MIC of isatin against *Asperillus niger* and *Candida albicans* was 156.25µg/ml. This study strongly iterates the medicinal value of the isatin.

Keywords: Antimicrobial activity, *Couroupita guianensis* Aubl., Isatin, Zone of inhibition.

INTRODUCTION

Couroupita guianensis Aubl. (Lecythidaceae) is commonly called Ayahuma and the Cannonball tree. It is an evergreen tree allied to the Brazil Nut (*Bertholletia excelsa*) and is native to tropical northern South America and the Southern Caribbean. Chemical studies of this species showed the presence of α-amirin, β-amirin, β-sitosterol, nerol, tryptanthrine, indigo, indirubin, isatin, linoleic acid, carotenoids and sterols.[1]

Isatin is one of the most frequently encountered heterocyclic in medicinal chemistry. It possesses various biological properties like antitumor, antimicrobial anti inflammatory, anti convulsant, antiviral, antioxidant, CNS depressant activities.[2]

MATERIALS AND METHODS

Test organisms

The bacterial and fungal strains used in the present study were the clinical isolates obtained from P.S.G Hospitals, Coimbatore. The bacterial strains used were *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri, Proteus vulgaris* and *Klebsiella pneumoniae.*

The fungal strains used were *Asperillus niger, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Mucor oryzae* and *Rhizopus indicus*.

Preparation of medium

The medium was prepared by dissolving 33.9g of the commercially available Muller Hinton Agar and potato dextrose agar medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto petriplates (25-30ml/plate) while still molten.

Preparation of the test culture

Inoculums of the microorganisms were prepared from overnight culture grown in nutrient broth and the suspension was adjusted with a turbidity equivalent to that of 0.5 MacFarland standard.

Preparation of test compound

The test compound was prepared at the concentration of 100mg/ml respectively in water.

Screening of antibacterial activity

Petriplates containing 20ml Mueller Hinton Agar medium were seeded, with the inoculums prepared from a broth that has been incubated for 6 hours, when the growth is in logarithmic phase, 100µl were spread in plates. Wells were cut in the agar and 10µl of the isatin was added in concentration of 100mg/ml. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by the diameter of zone of inhibition formed around the wells.[3] Amoxicillin was used as standard antibacterial agent.

Screening of antifungal activity

Petriplates with 20ml of Potato Dextrose Agar were prepared. A fungal plug was placed in the center of the plate. Sterile discs impregnated with the Isatin were placed in the plates. Nystatin was kept as positive control.

The growth was seen as a crescent shaped zone of inhibition.

Screening of minimum inhibitory concentration

Minimum Inhibitory Concentration was determined by micro dilution method using serially diluted sterile nutrient and potato dextrose broth (containing 2ml broth) for bacterial and fungal strains respectively to give concentration of 5000, 2500, 1250, 625, 312.5, 152.25 µg/ml. Microorganism in suspension, adjusted to turbidity to that of 0.5 MacFarland standards was added. These were incubated for 18 hours at 37°C. MIC was taken as the lowest concentration that did not give any visible growth.[4]

RESULTS AND DISCUSSION

Antimicrobial activity of Isatin

The Isatin was screened for its antimicrobial activity against different strains of bacteria and fungi and it was found that the Isatin showed zone of inhibition against all microorganisms. The Isatin showed the maximum zone of inhibition against *Proteus vulgaris, Escherichia coli, Staphylococcus aureus, Aspergillus flavus* and *Candida albicans*. The diameter of zone of inhibition for the isatin was shown in tables 1 and 2 and figures (1,2).

These findings are in agreement with previous study[5,6], who stated that the antimicrobial activity of extracellular metabolite of endophytic fungi, *Phomopsis* sp isolated from four medicinal plants (*Artabotrys odoratissimus, Cassia auriculata, Guazuma ulmifolia* and *Terminalia catappa)* against *E. coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Bacillus subtilis*. The antimicrobial screening of the extracellular metabolite showed varying zone of inhibition against the test organism varying from 2-13mm.

In the evaluation of the antifungal activity of 2H-Furo [2,3-H]-1-benzopyran-2-one isolated from seeds of *Psoralea corylifolia* L. they inhibited the fungal pathogens to the greatest extent of 90% inhibition.

Table 1: Antibacterial activity of Isatin by agar well diffusion method

Microorganisms	Diameter of zone of inhibition in mm	
	Isatin	Control (Amoxicillin)
Proteus vulgaris	S, 15.5±0.707	35
Escherichia coli	S, 14.5±0.707	35
Staphylococcus aureus	S, 14.5±2.121	30
Salmonella typhi	S, 14.5±2.121	34
Pseudomonas aeruginosa	S, 13±2.828	32
Klebsiella pneumoniae	S, 13±1.414	20
Shigella flexneri	S, 11.5±0.707	20

S- Sensitive.

Table 2: Antifungal activity of Isatin by agar plug method

Microorganisms	Growth Inhibition	
	Isatin	Control (Nystatin)
Aspergillus niger	++	+++
Aspergillus fumigatus	++	+++
Aspergillus flavus	+++	+++
Rhizopus indicus	++	+++
Mucor oryzae	++	+++
Candida albicans	+++	+++

+++ 100% inhibition, ++ ≤ 50% inhibition, + ≥ 50%, - - no inhibition

Minimum inhibitory concentration of isatin

Proteus vulgaris, Staphylococcus aureus and *Shigella flexneri* showed the appreciable MIC concentration of 312.5µg/ml whereas the MIC of isatin against *Asperillus niger* and *Candida albicans* was 156.25µg/ml. The minimum inhibitory concentration of isatin against bacterial and fungal strains was shown in tables 3 and 4.

Table 3: Minimum inhibitory concentration and minimum bactericidal concentration of Isatin

Microorganisms	MIC (µg)	MBC (µg)
Proteus vulgaris	312.5	312.5
Escherichia coli	625	625
Staphylococcus aureus	312.5	312.5
Shigella flexneri	312.5	312.5

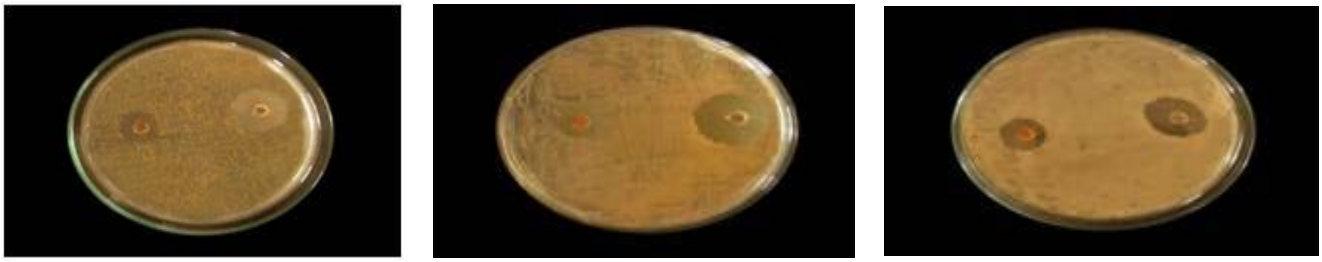
Table 4: Minimum fungicidal concentration of Isatin

Microorganisms	MIC (µg)	MFC (µg)
Aspergillus niger	156.25	156.25
Candida albicans	156.25	156.25
Rhizopus indicus	312.5	312.5
Mucor oryzae	312.5	312.5

The above results are in agreement with old literature[7,8], who found that the antibacterial activity of carvacrol, eugenol, linalool and 2-pentanoylfuran the essential oil obtained from plants, which inhibited the growth of the bacterial pathogens in a concentration dependent manner with MICs ranging between 0.25-2.5µg/ml. A study investigated the antifungal activity of cytotoxic rosane diterpenoid from *Hugonia castaneifolia* for determining the MIC, in which fungal growth was inhibited at a concentration of 12.5- 100µg/ml.

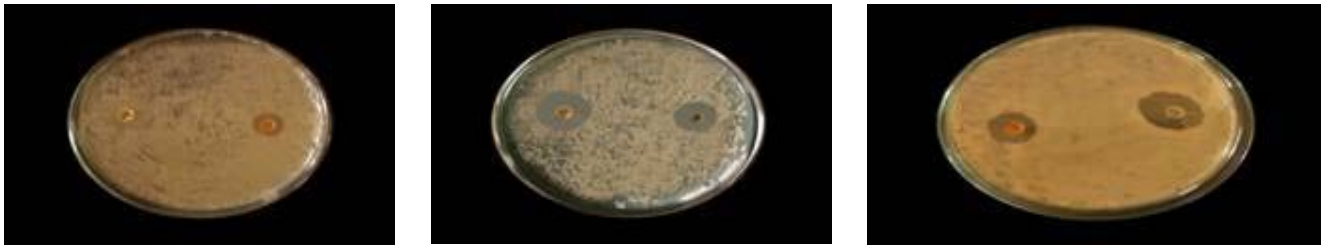
CONCLUSION

Thus, the present study strongly iterates the medicinal value of the Isatin and scientifically validates it for use as a component of medicinal preparations, to address the infectious disease caused by microorganisms.



Proteus vulgaris *Escherichia coli* *Staphylococcus aureus*

Figure 1: Antibacterial activity of Isatin



Aspergillus flavus *Candida albicans* I: Isatin; C: Control

Figure 2: Antifungal activity of Isatin

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