

## ANTIMICROBIAL ACTIVITY OF THE MYCOTOXIN CITRININ OBTAINED FROM THE FUNGUS PENICILLIUM CITRINUM.

PAPIYA MITRA MAZUMDER, RUPA MAZUMDER, A.MAZUMDER & D.S SASMAL

Department of Pharmaceutical Sciences

Birla Institute of Technology Mesra, Ranchi-83521 Jharkhand

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**ABSTRACT:** The mycotoxin Citrinin was obtained from the fungus *Penicillium citrinum*. It was tested for its Minimum Inhibitory Concentration (MIC) against some gram positive strains viz. *Staphylococcus aureus*, *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Lactobacillus arabinosus* and gram negative strains *E.Coli*, *Shigella dysenteriae*, *shigella sonnei*, *shigella boydii*, *Salmonella typhimurium*, *Proteus mirabilis* and *Vibrio cholerae*. Further the zones of inhibition produced by the fungal extract against the bacterial strains were assayed and compared with those produced by the standard antibiotic ciprofloxacin.

### Introduction:

The word Mycotoxin is derived from the Greek word **mykes** meaning fungus and the Latin word **toxicum** meaning poison<sup>(1)</sup>. Mycotoxins are generally defined as metabolites of fungi, which evoke pathological changes in man and animals<sup>(2)</sup>. The disease caused by mycotoxicosis<sup>(3)</sup>. The present investigations were undertaken to test whether there is any antimicrobial activity and to find out the MIC values of his fungal product against a series of Gram positive and Gram negative bacteria. Finally the antimicrobial potency of citrinin was assured by standard method against the same gram positive and gram negative strains and the results so obtained with the standard antibiotic Ciprofloxacin.

### Materials and Methods:

Pure culture of *Penicillium citrinum* MTCC 1751 was obtained from IMTECH Chandigarh. The inoculum was taken in sterile potato Dextrose Agar and fermented in a Rotary shaker at 28°C for 7days. The

Culture filtrate was made acidic whereby citrinin was precipitated which was subsequently extracted from chloroform and recrystallized from chloroform and recrystallized from absolute ethanol<sup>(4)</sup>. This toxin was detected by TLC<sup>(5)</sup>. The Citrinin thus obtained was tested for its antimicrobial activity against various bacterial strain like *staphylococcus aureus*, *Bacillus pumilus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Lactobacillus arabinosus* and gram negative strains *E.Coli*, *Shigella dysenteriae*, *shigella sonnei*, *shigella boydii*, *Salmonella typhimurium*, *Proteus mirabilis* and *Vibrio cholerae*. These bacterial strains were collected from the Dept. of Pharm. Tech. Jadavpur University, Central Drugs Laboratory, Kolkata, S.C.B. Medical College, Cuttack, Orissa and Institute of Microbial Technology (IMTECH), Chandigarh. All the strains used were pure culture preserved as stab slant cultures at a temperature of 4°C. The strains were

clinical isolates obtained from different parts of the country.

#### **Determination of Minimum Inhibitory Concentration (MIC) by Serial Dilution Technique (6).**

A Stock solution (25) ml of the mycotoxin of 1mg/ml concentration was prepared by dissolving 25 mg of the same in Dimethyl sulphoxide (**DMSO**). **DMSO** was taken in the control plate and did not show any activity as such. Calculated volumes of this stock solution were dispensed in a series of McCartney bottles previously containing calculated volumes of sterile cooled molten nutrient agar media (40°C- 45°C) to prepare the final volume of 20ml each, with dilutions 3,5,10,25,50,100,150 and 200 µg/ml. These molten nutrient agar media containing various concentrations of Citrinin were poured and solidified on to sterile petridishes to give sterile nutrient agar Plates with various concentrations of the toxin. After the plates were prepared they were kept in the refrigerator for 24hrs. for uniform diffusion of the fungal extract through the nutrient agar media. These plates were then dried at 37°C in the incubator for two hours prior to spot inoculation<sup>(6)</sup>. One loopful (loop diameter 3mm) of an overnight grown peptone water culture of each test organism served as the inoculum for the serial dilution technique. The back of each test plate was marked by checker board technique. The back of each test plate was marked by checker board technique for the location of each inoculums and the test organisms were spot inoculated accordingly. The spot inoculated accordingly. The spot inoculated plated were incubated for 24 hrs. at 37°C and the **MIC** values were determined.

#### **Determination of zones of inhibition by Disc diffusion Technique<sup>(7)</sup>.**

In this method, Ciprofloxacin pure was taken as the standard antibiotic for the comparison of the results. Of the results. Stock solutions (each of 1 mg/ ml concentration) of both the fungal toxin and the standard antibiotic were prepared. From these stock solutions, two sets of four dilutions (50,10,150,200 µg/ml ) each of citrinin (solvent **DMSO**) and Ciprofloxacin (solvent distilled water) were prepared in sterile McCartney bottles, Antimicrobial activity was determined by Disc Diffusion Assay Methods employing 24 hour peptone –water cultures of 27 test organisms. Sterile nutrient agar plates were prepared and incubated at 37°C for 24 hours to check for contamination if any.

Each sterile nutrient agar plate was then flooded with the corresponding peptone culture of the test organism, dried for 30 mins at 37°C and after drying of the flooded plate, four filter paper discs (Whatman no.1) of 6mm diameter were soaked in the four different dilutions of the crude extract and placed at the specific locations on the surface of the flooded plate, marked as quadrants at the back of the plates. The same technique was repeated in the case of the remaining test organisms for both the extract and the standard antibiotic. All the flooded plates with the corresponding filter paper discs on them were incubated at 37°C for 24 hours. The diameters of the zones of inhibition were measured in mm and compared accordingly.

#### **Results and Discussions:**

Results of the determination of determination of **MIC** values of the mycotoxin Citrinin have been recorded in Table-I it is quite evident for the results that Citrinin is active against both gram positive and gram negative bacteria. However from gram negative bacteria the reduction and

inhibition of growth start at lower concentration of the mycotoxin as compared to those in case of the gram positive strains. Thus, Table-1 depicts that the MIC values of citrinin are lower for Gram negative bacteria than antimicrobial potency of citrinin is further determined by standard Disc Diffusion assay technique <sup>(7)</sup> and results so obtained are compared with those of standard antibiotic Ciprofloxacin. The comparative results are recorded in Table – II. Further studies on in vivo antimicrobial

activity of Citrinin is going on in our laboratory and hope in future this mycotoxin will play an important beneficial role in the treatment of various microbial diseases provided it is having a high LD<sub>50</sub> value.

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**TABLE – 1**  
**DETERMINATION OF CITRININ AGAINST VARIOUS STRAINS OF BACTERIA**  
Growth in Nutrient agar containing diff concs. Of toxin (µg/ml)

<i>Name of Bacteria</i>	0+	3	5	10	25	50	100	150	200
<i>Shigella dysenteriae 1</i>	+	+	+	±	IC (6)	-	-	-	-
<i>Shigella dysenteriae 6</i>	+	+	+	±	IC (7)	-	-	-	-
<i>Shigella dysenteriae 2</i>	+	+	+	+	+	-	-	-	-
<i>Shigella</i>	+	+	+	+	+	-	-	-	-

<i>Sonnei 1</i>									
<i>Shigella boydii 8</i>	+	+	+	+	+	-	-	-	-
<i>Shigella Sonnei 2</i>	+	+	+	+	+	-	-	-	-
<i>E.coli ROW 7/12</i>	+	+	+	+	±	-	-	-	-
<i>E.coli AM 8/98</i>	+	+	+	+	±	-	-	-	-
<i>E.coli VC Sonawave 3.37C</i>	+	+	+	+	±	-	-	-	-
<i>E.Coli CD 99/1</i>	+	+	+	+	+	±	-	-	-
<i>Salmonella typhimurium ATCC 6539</i>	+	+	+	+	+	+	-	-	-
<i>Vibrio cholerae 865</i>	+	+	+	+	±	<b>IC (5)</b>	-	-	-
<i>Proteus sp. AM 8/98</i>	+	+	+	+	+	+	-	-	-
<i>Pseudomonas sp.</i>	+	+	+	+	+	+	-	-	-
<i>Staphylococcus Aureus M1267</i>	+	+	+	+	+	+	-	-	-
<i>Staphylococcus Aureus AM 8/98</i>	+	+	+	+	+	±	-	-	-
<i>Staphylococcus Aureus NCTC 7447</i>	+	+	+	+	+	±	-	-	-
<i>Staphylococcus Aureus 8531</i>	+	+	+	+	+	+	-	-	-
<i>Staphylococcus Aureus ATCC 29737</i>	+	+	+	+	+	±	-	-	-
<i>Bacillus pumilus 8241</i>	+	+	+	+	+	±	-	-	-
<i>Bacillus subtilis</i>	+	+	+	+	+	±	-	-	-
<i>Bacillus cerus var mycoides</i>	+	+	+	+	±	-	-	-	-
<i>Klebsiella Pneumoniae RM8/98</i>	+	+	+	+	+	+	+	-	-
<i>Streptococcus Pneumoniae NCTC 7465</i>	+	+	+	+	±	-	-	-	-
<i>Lactobacillus arabinosa CD/99/1</i>	+	+	+	+	+	±	-	-	-
<i>Sarcina lutea CD/99/1</i>	+	+	+	+	±	-	-	-	-

INDEX: 0+ Control plate without drug = Growth – No Growth

IC Isolated Colony ± Reduced Growth

**Table II**  
**ANTI – MICROBIAL ACTIVITY OF CITRININ**

Assay of antimicrobial potency of Citrinin and its comparison with the potency of standard antibiotic against the same strains of bacteria.

Name of Bacteria	Zone dia. In mm							
	Citrinin Conc. (µg/ml)				Ciprofloxacin Conc. (µg/ml)			
	50	100	150	200	50	100	150	200
<i>Shigella dysenteriae 1</i>	7.0	7.5	8.0	8.5	18.0	19.5	20.0	23.0
<i>Shigella dysenteriae 6</i>	7.5	8.5	10	10	10	111.5	12.5	14.0
<i>Shigella dysenteriae 2</i>	7	75	8.5	10	10	11.5	14.5	15.5
<i>Shigella Sonnei 1</i>	6.5	7.0	75	8.0	6.5	8.0	8.5	9.5
<i>Shigella boydii 8</i>	7	7.5	9.0	9.5	10.5	13.5	15.5	18.5
<i>Shigella Sonnei 2</i>	7	7.5	8.5	9.0	11.0	13.0	13.5	16.5
<i>E.coli ROW 7/12</i>	8	8	8.5	9.0	10.0	10.5	11.5	14.0
<i>E.coli AM 8/98</i>	6.5	7.5	8.0	85	6.5	8.0	10.5	11.5
<i>E.coli VC Sonawave 3.37C</i>	6.0	6.5	7.0	7.5	10.5	12.5	13.5	15.5
<i>E.Coli CD 99/1</i>	8.0	8.5	9.5	10	6.5	8.5	10.0	12.5
<i>Salmonella typhimurium</i>	6.5	7.0	7.5	8.5	11.0	11.5	14.5	15.5
<i>Salmonella typhimurium ATCC 6539</i>	7	8	8.5	9.0	9.0	11.0	12	13.5
<i>Vibrio cholerae 865</i>	6.0	6.5	7.0	8.5	8.0	8.5	9.0	10.0
<i>Proteus sp. AM 8/98</i>	6.5	7.5	8.5	9.0	6.5	8.0	12.0	14.0
<i>Pseudomonas sp.</i>	8.5	9.0	9.5	11	10.5	11.5	12.0	15.5
<i>Staphylococcus Aureus M1267</i>	6.5	70	75	8.0	11.0	15.5	16.0	20.0
<i>Staphylococcus Aureus AM 8/98</i>	6.0	6.5	7.0	75	7.5	10.0	12.0	13
<i>Staphylococcus Aureus NCTC 7447</i>	8.0	8.5	9.5	10	8.0	13.0	14.0	15.5
<i>Staphylococcus Aureus8531</i>	6.5	7.0	7.5	9.0	10.0	10.5	11.0	12.0

<i>Staphylococcus Aureus ATCC 29737</i>	6.5	7.0	7.5	8.0	8.0	10.5	13.0	13.5
<i>Bacillus pumilus 8241</i>	8.0	8.5	10.0	10.5	7.5	12.5	13.5	14.5
<i>Bacillus subtilis</i>	6.5	7.0	9.0	9.5	13.0	13.5	17.0	18.5
<i>Bacillus cerus var mycoides</i>	7.5	8.0	8.5	10	8.5	10.0	10.5	13.0
<i>Klebsiella Pneumoniae RM8/98</i>	8.0	8.5	9.0	10	10	12.0	14.5	15.0
<i>Streptococcus Pneumoniae NCTC 7465</i>	7.0	7.5	8.0	8.5	9.5	10.5	12.0	14.5
<i>Lactobacillus arabinosa CD/99/1</i>	6.0	6.5	7.0	15.0	9.5	11.0	11.5	14.0
<i>Sarcina lutea CD/99/1</i>	7.0	7.5	8.5	9.0	9.0	13.0	13.5	17.5