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Short Communication

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The Effect of Aurintricarboxylic Acid (ATA) and Copper-aurintricarboxylic Acid Complex (Cu-ATA) for Selective Inhibition on the Growth of Gram-positive and Gram-negative Bacteria

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Abstract

In this study, the antimicrobial activities of Aurintricarboxylic acid (ATA) and copper-aurintricarboxilylic acid complex (Cu-ATA) were evaluated against four bacterial species, both Gram-positive and negative; *Enterobacter aerogenes* (MTCC-111), *Clostridium perfringens* (MTCC-3296), *Enterococcus faecalis* (MTCC-5695), and *Vibrio parahaemolyticus* (ATCC-17802) using the Agar-well diffusion method. The results showed good antimicrobial activities for both the ligand ATA and Cu-ATA complex against Gram-positive bacteria compared to Gram-negative bacteria.

Keywords

Aurintricarboxylic acid, Copper (II) chloride, *Enterobacter aerogenes*, *Clostridium perfringens*, *Enterococcus faecalis*, *Vibrio parahaemolyticus*

Introduction

Foodborne diseases cause significant public health issues that result in social and economic burden [1-3] and in this content, copper complexes are known to reduce the number of food-borne pathogens caused by bacteria [4]. The temperature, characteristics of the copper complex, humidity, bacterial species, and type of contact between the bacteria and the surface of the copper, among other factors, can alter the antimicrobial activity of copper [5-7]. It has been hypothesized that copper ions released from the metal complex cause bacteria to damage their membranes, resulting in a loss of membrane potential and cytoplasmic content. Copper ion-produced reactive oxygen species also cause more cell structure damage and even DNA degradation [8, 9]. Because the reactive oxygen species values required for bacterial inactivation are below the toxicity threshold that is permitted for mammalian cells [10], copper applications can be used extensively in everyday life. In this paper, the anti-microbial activity of ATA and Cu-ATA complex on four types of bacteria *E. aerogenes, C. perfringens*, *E. faecalis*, and *V. parahaemolyticus* have been studied. All the above bacteria are observed to be pathogenic bacteria causing food-borne diseases or food poisoning. *E. aerogenes* causes spoilage in fish [11], *C. perfringens* is a lethal bacterium which easily spoils meat [12], *E. faecalis* causes infections in regularly consumed food like corn meal [13], and *V. parahaemolyticus* causes lethal sea food poisoning [14]. Therefore, determination of anti-microbial activity of ATA and Cu-ATA complex is important, and we have taken up a study of the effect of ATA and Cu-ATA complex on different types of bacteria. The results are reported in this paper.

Materials and Methods

Test microorganisms

The Bacterial cultures *E. aerogenes* (MTCC-111), *C. perfringens* (MTCC-

3296), *E. faecalis* (MTCC-5695), and *V. parahaemolyticus* (ATCC-17802) grown overnight at 37 °C were used for testing the antibacterial activity. These cultures were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India and maintained in freshly prepared nutrient agar slants. The organisms were preserved at -20 °C in the presence of glycerol (15%, v/v) for longer periods.

Antibacterial assay

The antibacterial activity of the various plant extracts on various bacterial strains was assayed by agar well diffusion method.

Nutrient agar medium (1 L)

The medium was prepared by dissolving 3.9 gm of the commercially available Nutrient Agar Medium-pH-6.8 (Hi-Media) in 100 ml of distilled water. The dissolved medium was sterilized in autoclave at 15 lbs pressure at 121 °C for 15 min, mixed well and poured onto 100 mm petri plates (25 - 30 ml/plate) while still molten.

Nutrient broth (1 L)

One liter of nutrient broth was prepared by dissolving 1.3 gm of commercially available Nutrient broth medium (Hi-Media) in 100 ml distilled water and boiling to dissolve the medium completely. The medium was dispensed as desired and sterilized in an autoclave at 15 lbs pressure (121 °C) for 15 min.

Chloramphenicol (standard antibacterial agent)

Nutrient agar medium (Hi-Media) was dissolved in water in 100 ml conical flask and was sterilized in an autoclave at 121 °C, 15 lbs for 15 min and poured in sterilized petri plates. Chloramphenicol was taken as a positive control for antibacterial activity. The antibacterial activity of ATA was evaluated by the agar well diffusion method [15]. Inoculum were spread over the surface of agar plates with a sterile glass spreader. Four wells were made at equal distances using a sterile cork borer. To test the antibacterial activity of ATA salt and Cu-ATA complex. It was made to a final concentration of 100 mg/ml. Aliquots of the plant extract (40 µg/ml, 60 µg/ml, and 80 µg/ml) were poured on each well and then plates were incubated for a period of 24 h at 37 °C in an incubator and the diameter (mm) of the clear inhibitory zone formed around the well was measured.

Results and Discussion

The antimicrobial activity for two different gram-positive bacteria *C. perfringens*, *E. faecalis* and two different gram-negative bacteria *E. aerogenes, V. parahaemolyticus* has been tested. The standard used in the test was Chloramphenicol which is used extensively in medication and has antimicrobial activity due to its inhibitory activity on protein synthesis. It is a broad-spectrum antibiotic known for its activity against anaerobic bacteria and on both gram-positive and -negative bacteria [16, 17]. Antimicrobial activity for both ATA and Cu-ATA complexes were checked with the same bacteria and standard

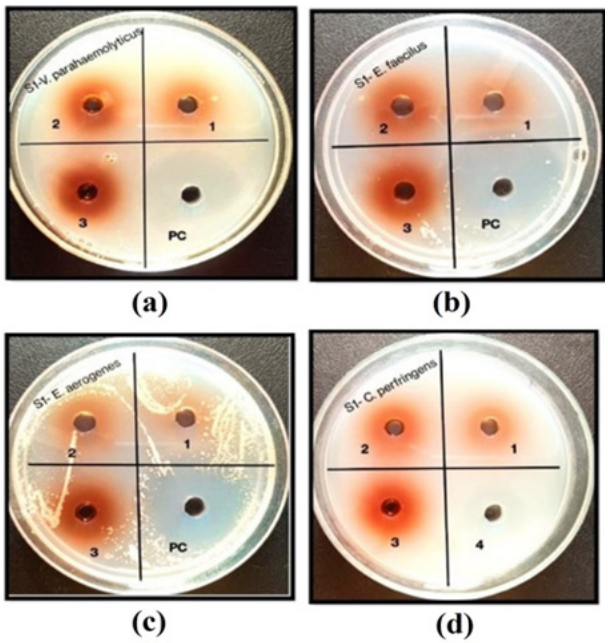

Figure 1: Antibacterial activity of ATA ligand on **(a)** *V. parahaemolyticus*, **(b)** *E. faecalis*, **(c)** *E. aerogenes*, and **(d)** *C. perfringens*.

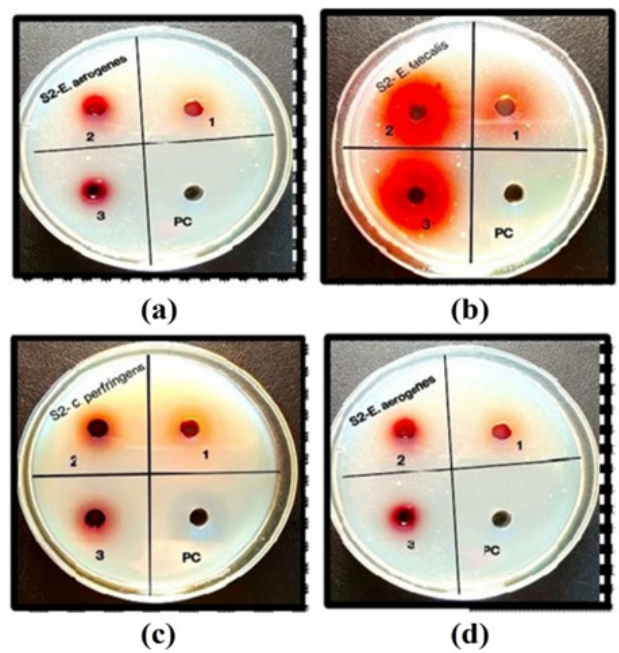

Figure 2: Antibacterial activity of Cu-ATA complex on **(a)** *V. parahaemolyticus*, **(b)** *E. faecalis*, **(c)** *E. aerogenes*, and **(d)** *C. perfringens*.

(Chloramphenicol). When compared with Chloramphenicol, ATA (Figure 1) and Cu-ATA (Figure 2) complex have almost similar.

Effect of concentration of ATA and Cu-ATA complex on antimicrobial activity

The study was carried out at three different concentrations, 40 µg/ml, 60 µg/ml, and 80 µg/ml of both ATA and Cu-ATA complex. Among the three concentrations, the inhibition was high at 80 µg/ml. It was found that the Cu-ATA complex is more effective compared to ATA in amicrobial activity.

Table 1: Antibacterial activity of Aurintricarboxylic acid salt against selected bacteria.

Test Organism	Zone of Inhibition (mm)			
	ATA (S1) (µg/ml)			Standard (Chloramphenicol) 20 µg/ml
	40	60	80	
***Enterobacter aerogenes* (MTCC-111)**	-	12	14	20
***Clostridium perfringens* (MTCC-3296)**	8	10	14	14
***Enterococcus faecalis* (MTCC-5695)**	12	14	18	29
***Vibrio parahaemolyticus* (ATCC-17802)**	10	12	16	24

Table 2: Antibacterial activity of Cu-ATA complex sample against selected bacteria.

Test Organism	Zone of Inhibition (mm)			
	Cu-ATA (S1) (µg/ml)			Standard (Chloramphenicol) 20 µg/ml
	40	60	80	
***Enterobacter aerogenes* (MTCC-111)**	-	8	10	24
***Clostridium perfringens* (MTCC-3296)**	10	12	13	16
***Enterococcus faecalis* (MTCC-5695)**	-	10	20	22
***Vibrio parahaemolyticus* (ATCC-17802)**	12	14	18	22

Compared to the Cu-ATA, ATA was showing a weak zone of inhibition for bacteria *E. faecalis* and *V. parahaemolyticus* in all concentrations. For *C. perfringens* the zone of inhibition was the same for both ATA and Cu-ATA complex. Both performed weakly against *E. aerogenes* when compared with the standard. (Table 1 and Table 2).

Effect of ATA and Cu-ATA complex on gram-positive and gram-negative bacteria

The study was carried out on the metal-ligand complex was effective on Gram-positive bacteria rather than Gram-negative bacteria. When the species are exposed to bacteria cultures, the ability to cross the cell membrane of Gram-positive bacteria is easier compared to Gram-negative bacteria. This is because the Gram-negative bacteria have an additional outer membrane that will protect it more from anti-microbial agents [18, 19]. The treatment might enhance the lipophilic character of the Cu-ATA complex compared to the ATA which increases the permeability for crossing the cell membrane. The other factor that makes the metal-ligand complex a powerful bactericidal agent is a chelation which is superior to an uncoordinated ligand or individual metal ion [20].

Thus, the Cu-ATA complexes have more zone of inhibition to *E. faecalis*, and more or less equal zone of inhibition in *C. perfringens* when compared with *V. parahaemolyticus* which is a Gram-negative bacterium.

The other reason for antibacterial activity on bacterial strains can be due to the copper-dependent peroxidation of unsaturated fatty acids in the cytoplasmic membrane which kill the bacteria. Copper-induced hydroxyl radical formation, according to this model, leads to lipid peroxidation, which causes immediate membrane depolarization, inhibition of respiration, and ultimately cell death [21].

Conclusion

ATA and Cu-ATA complex was observed to be effective against Gram-positive bacteria and selectively effective against Gram-negative bacteria. The reasons can be attributed to the ability of the complex to penetrate the cell wall and the toxicity caused by copper to damage cell walls and cell components. The zone of inhibition is high at 80 µg/ml concentration for both gram-positive and gram-negative bacteria. These findings may help in the utilization of ATA ligand and Cu-ATA complexes in the fields of the food industry, medical equipment, agriculture, and pharmaceutical industry.

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Conflict of Interest

There are no conflicts of interest to declare on behalf of the authors of the manuscript.

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