Antimicrobial potential of mixed ligand copper(II) complexes with dimethylglyoxime and some amino acids

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Summary

In this work, six copper(II) complexes with dimethylglyoxime and amino acids (tryptophan, glutamate, proline, arginine and valine) have been prepared and provided by coordination chemistry laboratory from Mammeri University (Algeria) to be tested in vitro for their antimicrobial activities. The copper(II) complexes identified by numbers 1 to 6 were found to be solid, insoluble in water, ethanol, and methanol, but soluble in dimethylsulfoxide, and dimethylformamide. The antimicrobial activity of the ligands and their metal complexes were tested against the *Escherichia coli* (Gram negative bacteria), *Bacillus cereus* (Gram positive bacteria), *Candida albicans* (yeast) and *Aspergillus niger* (mold). The determination of the antibacterial activity was performed using well agar diffusion method. Results obtained demonstrated that only complexes 2 and 6 were active against bacteria compared to ampicilline (antibiotic) used as positive control. Whereas complexes 2, 3 and 5 tested against *Candida albicans* and *Aspergillus niger* were shown more active against *C. albicans* than *A. niger*. Our findings suggested the possibility of use of copper(II) complexes as antimicrobials in food preservation or cleaning products.

Key words: copper(II) complexes, dimethylglyoxime, amino acids, antimicrobial activity

Introduction

Copper (CuII) complexation was reported to have biological proprieties and to be extensively used in clinical applications such as enzyme inhibition agents [1] and antimicrobials [2]. Copper complexation may be performed with amino acids: essential elements of proteins involved in biochemical processes in living organisms. One of interesting proprieties of these complexes is their chelating potential and capacity to coordinate transition metals through their amino or carboxylic groups (tryptophan, arginine, proline valine and glutamate)[3]. Currently, copper complexes with amino acids are gaining more attention due to their potential use as antibacterial agents. Dimethylglyoxime is a potentially tetradentate ligand as well as a mono, bi and tri-dentate ligand, and copper complexes with dimethylglyoxime are known for their high stability. However, the biological activity of these compounds was poorly investigated.

This work aimed to assess antimicrobial activity of six new copper(II) complexes produced from dimethylglyoxime as primary ligand and a several amino acids, as secondary one. All complexes obtained and their ligands were screened for antibacterial activity against Gram-negative (*Escherichia coli*), Gram-positive (*Bacillus cereus*), antifungal activity against yeast (*Candida al*bicans) and mold (*Aspergillus niger*).

Materials and Methods

Cupper complexes

Six new copper(II) complexes with dimethylglyoxime and amino acids (tryptophan, glutamate, proline, arginine and valine) were synthetized and purified at M. Mammeri university, and characterized by elemental microanalysis carried out at the Service of Microanalysis, Faculty of Pharmacie-University Paris-Sud, France.

Antimicrobial test

Copper complexes synthesized and their ligands were tested against pathogenic bacterial and fungal species. The antimicrobial potential of complexes was compared to those of natural substances such as thyme essential oils (*Thymus vulgaris*) (FLORAME, France) and commercially available citric acid. Copper complexes and their ligands (10 mg/ml), as well as thyme essential oils were dissolved and diluted in DMSO.

Ampicillin (AMC) and DMSO were used as positive and negative control, respectively. Antimicrobial activity was assessed using agar diffusion method (in Muller Hinton) against *Escherichia coli* ATCC 8739 and *Bacillus cereus* ATCC 6633, *Candida albicans* ATCC 10231, and *Aspergillus niger* [4].

Inoculation was achieved at 10^6 CFU/ml using cultures grown overnight at 30 °C, and incubation was performed at 24 h (Bacteria) or 72 h (fungus) at 30 °C. Diameters of inhibition zones (Φ) were measured in millimeters, and sensitivity to antimicrobials was classified as following: not sensitive (Φ<8 mm); sensitive (Φ: 9-14 mm); very sensitive (Φ:15-19 mm); extremely sensitive (Φ > 20 mm) [5].

Results and Discussion

Values of inhibition zone diameter obtained by copper complexes and their ligands tested against *E. coli* and *B. cereus* are shown in Figure 1.

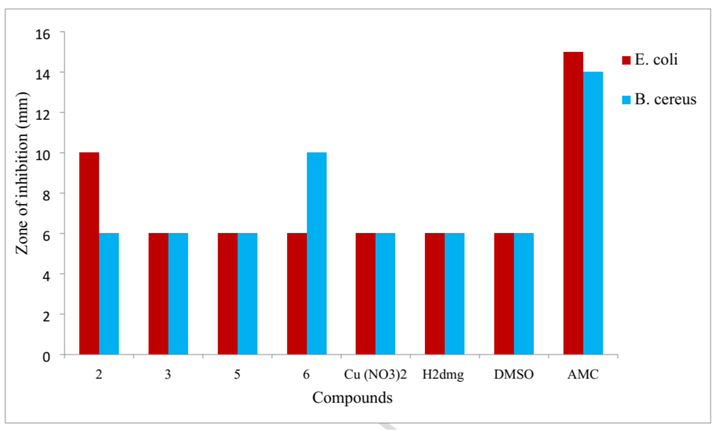

Figure 1. Values of inhibition zone diameter (mm) obtained by copper complexes and their ligands tested against *E. coli* and *B. cereus*. DMSO (solvent) and ampicillin (antibiotic) were used as negative and positive control respectively.

Results obtained demonstrated that only complexes 2 and 6 were active against both bacteria tested with comparison to antibiotic ampicillin (positive control). Gram negative bacteria *E. coli* was revealed sensitive (Φ: 10 mm) to the complex 2, whereas the Gram positive bacteria *B. cereus* was inhibited by complex 6 (Φ: 10 mm). However, all ligands used were shown inactive against both bacteria tested.

The inhibitory effect observed could be explained by neutrality of complexes 2 and 6, which was reported to increase lipophilic propriety enabling their penetration through lipid layer of bacterial membranes and leading to enzymatic inhibition, DNA bonding, respiration and protein synthesis disruption in microorganisms [6].

Results displaying antifungal activity of antimicrobials tested are presented in Figure 2.

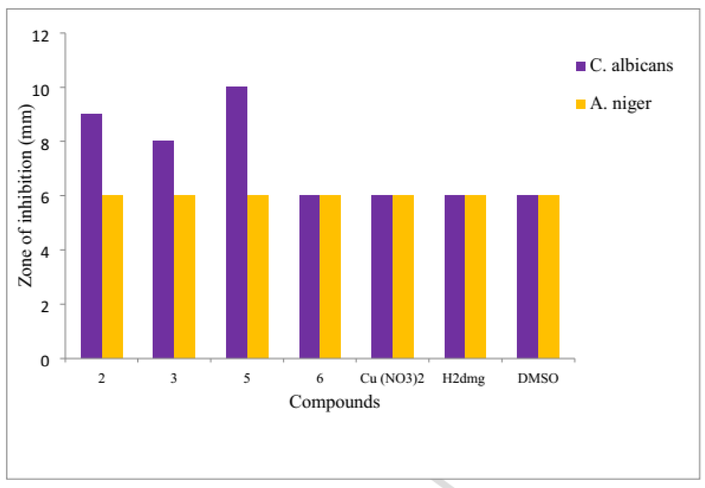

Figure 2. Values of inhibition zone diameter (mm) obtained by copper complexes and their ligands tested against *C. albicans* and *A. niger*. DMSO (solvent) was used as control negative.

Copper complexes 2 and 5 demonstrated a fungistatic effect against C. *albicans* (Φ: 9 to 10 mm), while a weak inhibitory effect was observed with complex 3 on the same fungal strain. Our observations are similar to those of Urquiza et al. [7], demonstrating that copper complexes inhibit *C. albicans*.

No effects were exhibited by ligands and DMSO tested against *Candida albicans* and *Aspergillus niger*, that means complexation was necessary for antifungal activity of compounds tested.

Glutamate or arginine in complexes 2, 3 and 5 are amino acids with a charged side chain enabling them to have electrovalent bonding with certain constituents of yeast membrane (proteins in particular) and altering membrane permeability and enzymatic activity in cytoplasm.

Results displaying comparison between antimicrobial activities obtained by copper complexes 2, 3, 5, and 6 versus natural compounds commercially available are shown in Figure 3.

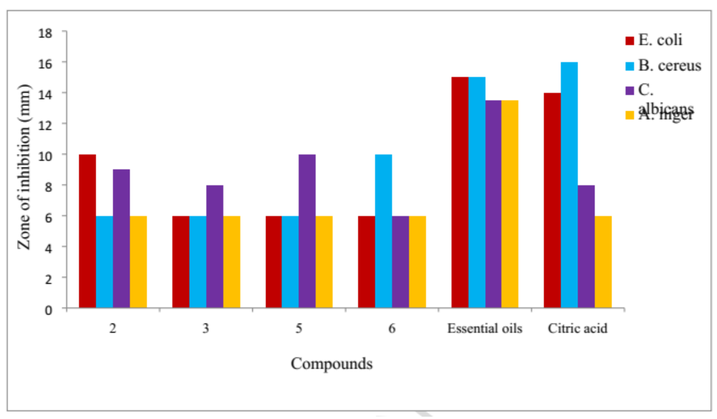

Figure 3. Comparison between antimicrobial activities obtained by selected copper complexes versus natural compounds commercially available. Complexes 2, 3, 5, and 6 were tested at the same concentration (10 mg/ml), while thyme essential oils and citric acid were diluted at optimal ratio 1:8 and 1:10 (v:v) respectively.

Results obtained demonstrated that antimicrobial activity of complexes 2, 3, 5 and 6 is comparable to that of natural compounds (thyme essential oils and citric acid). These results indicated that sensitivity of both bacteria and fungi to copper complexes is comparable to those of essential oil and citric acid.

Thyme essential oils were found more active than citric acid and complexes synthetized. Thyme essential oils were reported to inhibit significantly the growth of several bacterial species: *Escherichia coli*, *Salmonella Typhimurium*, *Staphylococcus aureus* [8].

Conclusions

Overall results obtained demonstrated that only copper complexes with glutamate or valine were active against bacteria compared to control. Whereas complexes with glutamine and arginine were more active against *C. albicans* than *A. niger*. Antimicrobial activity of complexes synthetized was probably due to chelation / coordination process causing alteration of cellular function. Ours findings suggested the possibility of use of copper complexes as specific antibacterial agents or sanitizers.

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