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Synthesis of Curcumin - Copper Complex and Assessment of its Antimicrobial Potential

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Abstract
Curcumin is a bioactive component obtained from the rhizome of turmeric plant. Curcumin forms strong complexes with several metal ions, including boron, cobalt, copper, gold, manganese, nickel, iron, silver, zinc, etc. Metal–curcumin complexes have been found to have multiple biological activities including antimicrobial potential. In the present study, curcumin – Copper (Cu) complex was synthesized and tested for antimicrobial activity against four bacterial strains such as *Bacillus* sp., *Pseudomonas* sp., *Salmonella* sp. and *Vibrio cholerae* and a fungal strain, *Candida* sp. These microbial strains are known to cause diseases in humans as well as animals. The curcumin-Cu complex exhibited significant growth inhibitory potential against all the strains tested. The curcumin-Cu complex could be an appropriate candidate for additional *in vivo* investigations.

Keywords: Curcumin, Copper, Metal complex, curcumin-Cu complex, Antibacterial activity, Antifungal activity

INTRODUCTION

Bacterial resistance to most of the available antibacterial agents is a growing problem worldwide. Thus the development of novel antibacterial agents that could overcome the resistance problem has become the major subject of ongoing research [1]. Plant-derived antimicrobial agents have proved to be promising alternatives to conventional antibiotics [2]. Turmeric (*Curcumin longa* L.) is a perennial herbaceous plant that reaches a stature of up to 1.0 meter. They produce highly branched, yellow to orange, cylindrical, aromatic rhizomes [3], which are of great economical value. Curcumin is a naturally occurring poly phenolic phytoconstituent isolated from the rhizomes of *C. longa* and is an important permitted natural colorant in food and pharmaceutical preparations [4].

Curcumin is used as a spice in many of the Asian countries as a flavor and color in food and also as a traditional medicine against a lot of diseases [5]. Curcumin is reported to have antioxidant, anti-inflammatory, antibacterial, antifungal, and antiviral activities [6]. In spite of having multiple therapeutic potentialities, curcumin is still not considered as a good therapeutic agent owing to its chemical instability, poor bioavailability, rapid metabolism and poor absorption. Complexation of curcumin with transition metals forming metal-curcumin complexes has been found to increase the solubility and cellular absorption, and thereby the bioavailability of curcumin [7]. Copper is one of the most important trace elements involved in human metabolism. It is an essential element, necessary for sustaining life [8, 9]. Copper is reported to have biological activities including antimicrobial potential [10, 11].

Microbial strains such as *Bacillus sp*., *Pseudomonas* sp., *Salmonella* sp., *Vibrio cholerae* and *Candida* sp., were used in the current study. Strains of these microbes have been reported to be pathogens of both humans as well as animals. Species of *Bacillus* are known to cause anthrax, bacteraemia, septicaemia and mastitis in animals [12]. *Pseudomonas* found in soil and water colonize plants and animals including man and a few species are pathogenic to animals and humans [13]. *Salmonella* is an important pathogen of economic importance in animals and humans. The prominent reservoir of *Salmonella* is the intestinal tract of a wide range of domestic and wild animals, being very significant in poultry [14]. *Vibrio cholera* that widely exists in various water bodies **cause** infection in aquatic animals [15]. *Candida* causes a fungal disease in animals called candidiasis [16].

Review of literature indicated that the studies on antimicrobial activities of **Curcumin**-Cu complex are limited [11, 17, 18]. Not all the strains employed in this study have been tested in the previous studies and also the strains that were used are different from the ones employed in the present study. Different strains of the same bacterial species are known to differ in their resistance or susceptibility to antimicrobial agents [19]. Owing to this, the present study was carried out to

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synthesize curcumin-Cu complex and to evaluate its antimicrobial activity *in vitro* against four bacteria and a yeast stain.

MATERIALS AND METHODS

Synthesis of curcumin

Synthesis of the complex was carried out according to Gubendran et al. [11] with slight modifications. The curcumin-Cu complex was synthesized by mixing equal volume of solutions of copper (II) nitrate (0.375 g, 2.0 mmol) and curcumin (1.47 g, 4.0 mmol) in ethanol and the mixture was heated at 60°C for 1 h in nitrogen atmosphere. Molar ratio of curcumin to copper was 1:2. The reaction was further continued for 2 h under reflux at room temperatue. Solid complex that was produced was separated by filtration and washed several times with 1:1 water and ethanol combination, to remove unreacted curcumin and copper, finally vacuum-dried.

Antimicrobial activity assay

Microbial strains such as *Bacillus* sp., *Pseudomonas* sp., *Salmonella* sp., *Vibrio cholerae* and *Candida* sp., were used for the study. Stock solution of curcumin-Cu complex (10 mg / ml = 10000 ppm) was prepared by dissolving it in dimethyl sulphoxide (DMSO). Using this stock solution all the concentrations tried, 500 ppm (0.5 mg/ml) to 5000 ppm (5 mg/ml), were prepared by diluting with distilled water. Antibacterial and antifungal activity assay was carried out employing the agar well diffusion method on nutrient agar (NA) and potato dextrose agar (PDA) medium, respectively. 24-h-old broth cultures were used as inoculum. Sterile NA and PDA plates were prepared. 20 ml of sterile medium was poured in to sterile 90 mm Petri plates and allowed to solidify. A lawn of inoculum was made on the solidified media in the plates using sterilized cotton swabs and allowed as such for few minutes. Using a 5-mm cork borer wells were made equidistantly on the medium. 100 µl of different concentrations of curcumin-Cu complex were added to different wells. 100 µl of DMSO served as the control that was added to the central well in the plate. All bacterial test plates were incubated at 37° for 24 h and for fungi it was extended up to 48 h. After incubation the plates were observed for the zone of inhibition, which was measured and noted down. The experiment was repeated twice with three replicates each time.

RESULT AND DISCUSSION

The curcumin-Cu complex exhibited significant antibacterial activity against both Gram positive and Gram negative bacteria, with better activity against Gram negative bacteria than Gram positive bacteria. It also exhibited substantial antifungal activity against *Candida* sp. Susceptibility of the pathogens varied with the concentrations of the complex wherein antibacterial activity of complex increased with the concentration. The pathogens showed varied sensitivity to curcumin-Cu complex (Fig. 1; Table 1). Comparatively *Salmonella* sp. and *Psuedomonas* sp., were more susceptible while *Vibrio cholerae*, was least susceptible, considering the amount of inhibition observed. Curcumin-Cu complex exhibited comparatively better activity than curcumin at 5 mg/ml concentration except *Vibrio cholerae* (Table 2).

Table 1: Antimicrobial activity of curcumin-Cu complex against some human pathogenic microbes at different concentrations

Test organism	**Zone of inhibition in mm**					
	0.5 mg/ml	**1 mg/ml**	**2 mg/ml**	**3 mg/ml**	**4 mg/ml**	**5 mg/ml**
Gram positive bacteria						
Bacillus sp.	NT	6.3 ± 0.66	8.6 ± 0.80	9.3 ± 0.71	10.6 ± 0.66	12.0 ± 0.57
Gram negative bacteria						
Pseudomonas sp.	NT	10.6 ± 0.61	11.3 ± 0.80	11.6 ± 0.76	12.0 ± 0.85	13.0 ± 0.57
Salmonella sp.	NT	9.3 ± 0.55	12.3 ± 0.66	13.6 ± 0.91	16.6 ± 0.61	18.3 ± 0.84
Vibrio cholerae	NT	7.0 ± 0.68	8.3 ± 0.55	9.0 ± 0.57	9.3 ± 0.76	10.3 ± 0.76
Fungus (Yeast)						
Candida sp.	9.5 ± 0.67	16.0 ± 0.95	17.3 ± 0.80	18.0 ± 0.93	NT	NT

Values are means of two experiments, each with triplicates ± SE; NT – Not Tested

Table 2: Antibacterial activity of curcumin against some human pathogens at different concentrations

Test organisms	**Zone of Inhibition in mm**				
	1 mg/ml	**2 mg/ml**	**3 mg/ml**	**4 mg/ml**	**5 mg/ml**
Gram positive bacteria					
Bacillus sp.	0.0	0.0	6.0 ± 0.31	6.8 ± 0.48	10.5 ± 0.62
Gram negative bacteria					
Pseudomonas	0.0	0.0	8.0 ± 0.52	9.6 ± 0.33	10.6 ± 0.56
Salmonella sp.	0.0	0.0	8.5 ± 0.56	11.5 ± 0.43	15.2 ± 0.60

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Vibrio cholerae	0.0	0.0	9.3 ± 0.52	10.6 ± 0.33	12.0 ± 0.56

Values are means of two experiments, each with triplicates ± SE

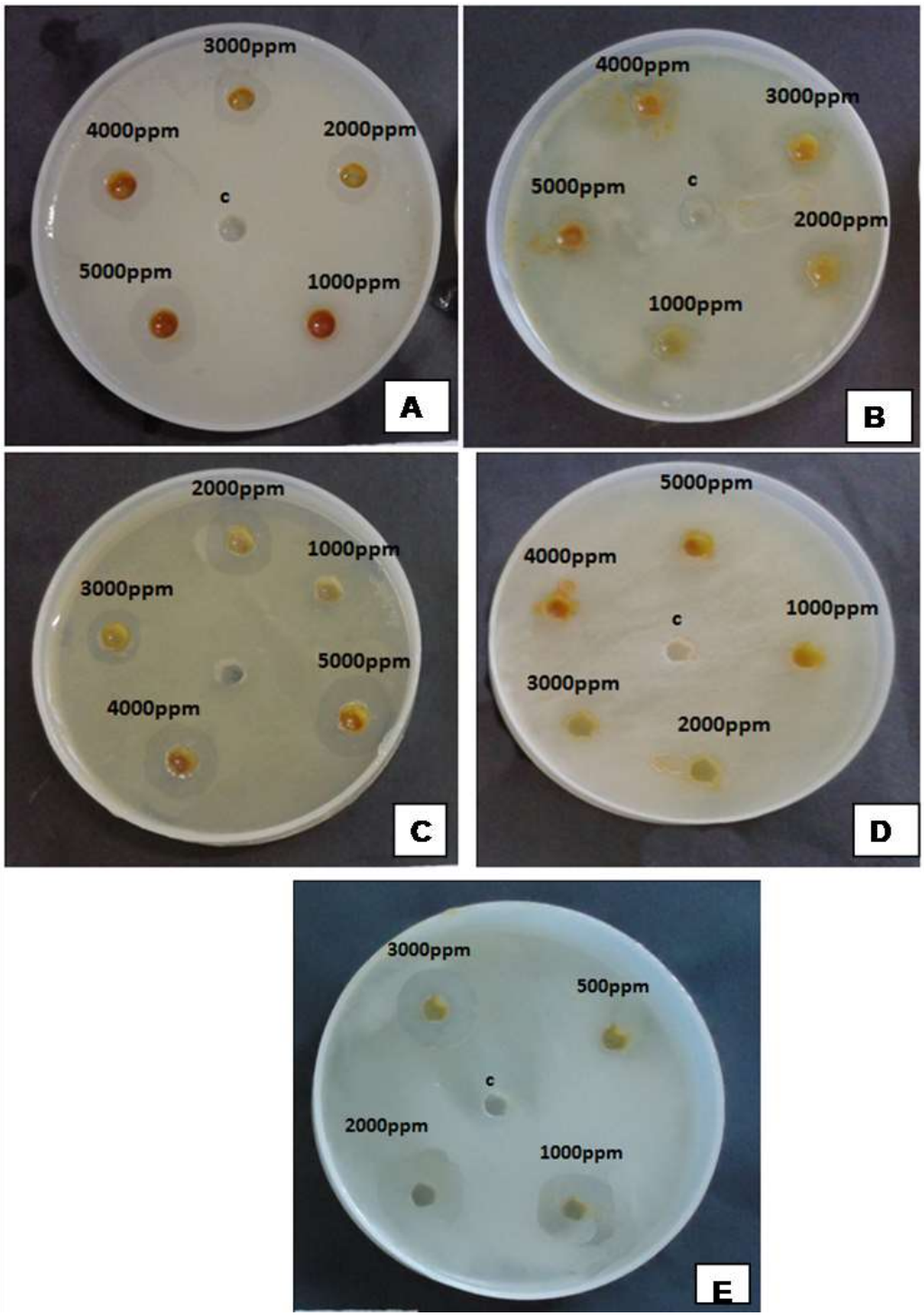

Figure 1: Antimicrobial activity of curcumin-Cu complex against (A) *Bacillus* sp., (B) *Pseudomonas* sp. (C) *Salmonella* sp. (D) *Vibrio cholerae,* and (E) *Candida* sp. at different concentrations

Curcumin acts as a ligand and forms stable complex with metals such as copper. Copper is reported to have several therapeutic properties including marked antimicrobial activities [20]. Curcumin is well known to have antioxidant, anti-inflammatory, anticancer, anti-microbial, antidiabetic, neuroprotective, immune boosting and other therapeutic properties. However, efficacy of curcumin is restricted because of water insolubility, instability, low intestinal absorption leading to its poor bioavailability and systemic elimination [21]. The complexing of curcumin with metals increases its water solubility and stability and subsequently efficacy of curcumin [7].

In the present study, curcumin-copper complex was prepared and studied for antibacterial activity against four bacterial strains such as *Bacillus sp., Pseudomonas* sp., *Salmonella* sp. and *Vibrio cholerae* and antifungal activity against *Candida* sp., by agar well diffusion assay. The complex exhibited substantial activity against all the bacteria and a yeast strain tested. This is in accordance with the observation of a few other studies. Curcumin copper complex substantially inhibited the growth of bacteria in the order *Klebsiella pneumoniae* > *Escherichia coli* > *Staphylococcus aureus* > *Pseudomonas fluorescence* [11]. Curcumin-Cu complex exhibited very good inhibition of *E. coli, Klebsiella* sp., and *C. albicans*, while there was no inhibition of *S. aureus and Streptococcus mutans* [17]. Curcumin-Cu showed significant activity more than curcumin against *E. coli* and *S. aureus* [18]. Curcumin complexes with Co^{2+}, Ni^{2+}, and Cu^{2+} ions exhibited good antibacterial activity against *S. aureus, E. coli, K. pneumoniae, P. aeruginosa* and *Streptococcus pyogenes*. The Cu^{2+}–curcumin complex showed the highest antibacterial activity [22].

Literature survey indicates that probably this is the first report of antibacterial activity of curcumin-copper complex against *Bacillus and Vibrio cholerae*. Degradation efficiency of curcumin-metal complex inside the cells is higher that increases bioavailability of curcumin and complexation of curcumin with metals also increases pharmacological effects of curcumin [7]. This might be the reason for increased antimicrobial activity of the complex compared to that of curcumin observed against most of the microbes tested in this study.

CONCLUSION

Complexation of curcumin with copper improves the aqueous solubility, stability and bioavailability of curcumin. Curcumin-Cu complex showed significant antimicrobial activity and could be a suitable candidate for further *in vivo* investigations.

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