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Efficacy of Copper Charged Water against *Candida albicans*

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Abstract

Copper has been proved to be an effective antimicrobial agent with added advantage of being harmless to human at low concentration. This research has been done to assess the affectivity of copper along with some other metals, on an opportunistic pathogen *Candida albicans*. Copper was found to be the most potent of all. Metallic copper was then compared with copper charged water for its action on the same fungus. Along with this brilliant metal, its water also has antifungal action even better than a standard antifungal drug, fluconazole, at its MIC. Upon introduced in media, copper charged water shows changes in Candida growth pattern.

Keywords: Copper charged water; Conductivity, *Candida albicans* NCIM 3471; Time point killing assay; Atomic emission spectroscopy; Growth kinetics

Introduction

Copper was the first element to be discovered in metallurgy. Copper and its alloys, specifically brass and bronze were used in ancient India for making statues, utensils, ornaments, coins etc. In India, copper was used to sterilize drinking water in 2600 B.C. and 2200 B.C. It is still used in many households for storing water.

Today copper is used as a water purifier, algaecide, fungicide, nematocide, and molluscicide and as an anti-bacterial and anti-fouling agent. Copper also displays potent anti-viral activity. Copper is considered safe to humans, as demonstrated by the widespread and prolonged use of copper intrauterine devices (IUDs) by women [1-3]. In contrast to the low sensitivity of human tissue (skin or other) to copper, microorganisms are extremely susceptible to copper. Studies have shown that copper can destroy orinhibit the growth of various bacterial strains. Antimicrobial copper, brass and bronze surfaces kill greater than 99.9% of bacteria within 2 hours of exposure [4]. Alloys with higher copper content kill organisms faster. It also boosts the immune system and prevents excess energy from being expended fighting off the infections.

Ayurveda recommend storing of water in copper vessels. When water is stored in a copper vessel, the copper gently leaches into the water and lends it all its positive properties. Drinking water stored in a copper vessel is beneficial to our health. According to Ayurveda, water stored in a copper vessel has the ability to balance all the three *doshas* in our body, (*vata, kapha* and *pitta*) and it does so by positively charging the water. The water stored in a copper vessel is known as '*tamarajal*' and is supposed to be consumed after storing the water in a copper vessel for at least eight hours. Copper is an essential nutrient for humans as well as bacteria but, in high doses, copper ions can cause a series of negative events in bacterial cells. Copper's rate of microbial inactivation can be affected by temperature, copper concentration and the type of microorganism with which it is in contact. The mechanisms of antimicrobial action by copper and its alloys, including brass, is a subject of intense and on-going investigation.

Today, the antimicrobial uses of copper have been expanded to include fungicides, pesticides, antimicrobial medicines, oral hygiene products, hygienic medical devices etc. The fungal infections are not usually dangerous, although it can cause discomfort, may be resistant to treatment, and may spread to other parts of the body or other people. Affected feet can also become secondarily infected by bacteria. Opportunistic pathogens of human are the most common cause of infectious disease–related deaths in the United States [5]. They are the causes of variety of invasive or systemic fungal infections in immuno-compromised or immunosuppressed individuals. Pathogens like, *Candida, Fusarium, Trichosporon*, and *Malassezia* species are emerging as important nosocomial pathogens.

Approximately 90% of human fungal infections are caused by *Aspergillus, Candida, Cladosporium, Epidermophyton, Microsporum* and *Trichophyton* species**.** These have become one of leading cause of death among patients due to greater use of broad spectrum antibiotics, immunosuppressive agents, and intensive care of low birth weight infants, organ transplantation and the acquired immunodeficiency syndrome (AIDS) epidemic**.** The situation is alarming in developing as well as developed countries like India, due to indiscriminate use of antibiotics. For instance, 33% of late-stage AIDS patients in one study had drug-resistant strains of *Candida albicans* in their oral cavities [6]. Hence, infection with this fungus is a major problem among immune-compromised patients (such as AIDS patients). For years, practices are being carried out to fight against these pathogens. Use of antimicrobial drugs such as broad spectrum antibiotics, herbal formulations, Ayurvedic medications such as decoctions; *Bhashma*, etc. are amongst them.

Currently used antifungal agents

There are many antifungals used currently, like Amphotericin B, Fluconazole, Itraconazole,Caspofungin etc. But these antifungal antibiotics are becoming ineffective leading to resistant strain development and various other drawbacks in terms of, for example, toxicity, drug–drug interactions and high cost [7]. Apart from side-effects like liver damage or affecting oestrogen levels, many antifungal

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medicines can cause allergic reactions in people. The scientific literature cites that copper kills microorganisms by multiple pathways rather than by acting in a specific way on one receptor. Hence, traditionally used copper charged water can be an interesting option to be analysed against pathogenic fungi. As copper is a natural mineral required by our body, it will also help in avoiding the adverse effects of already used antifungal drugs.

Materials and Methods

Sample preparation and analysis

A copper vessel of 99% purity was bought from a local market of Guwahati, Assam. Sterile demineralized water of known volume was kept in the cleaned vessel for 24 hrs and aliquots were taken out at regular intervals for analysis. Surface to volume ratio of the vessel was calculated for the vessel. Conductivity and pH of the copper charged water were measured after 24 hrs. The aliquots were subjected to analysis by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), IIT Bombay, for the evaluation of copper elution rate from the vessel.

Micro-organism and culture conditions

Candida albicans NCIM 3471 (ATCC 10231) culture was purchased from NCIM, Pune. The strain was grown on potato dextrose agar media and incubated at 35°C for 48h before use.

Media and chemicals

Potato dextrose agar (PDA) and broth (PDB) were bought from HiMedia. Standard fluconazole (ZOKON-50) 50 mg tablets were used in the experiment.

Effect of metals

Candida suspension was prepared using sterile distilled water from a 48 hr old culture. Equal volumes of the suspension containing 1.5×10^4 cells/ml were kept in sterilized vessels of steel, aluminium, iron, brass and copper. Sterilized glass conical flask was used as control. All of them were incubated at room temperature (RT) for 24 hrs. 100 µl of each suspension was evenly spread onto sterile PDA plate, incubated for at RT for 48 hrs. CFU/ml was calculated and affectivity was checked.

Time-point killing assay

1.5×10^4 cells/ml *Candida* culture was inoculated in copper charged water, copper vessel, fluconazole (32 ppm) and in Phosphate Buffered Saline (PBS). 100 µl aliquots from each were plated onto sterile PDA at 0 min, 60 min, 120 min, 180 min and 24 hr. after incubation for 48 hr at RT, the CFU/ml was calculated.

Growth kinetics

Growth curve was studied by inoculating 1×10^4 cells/ml in sterile PDB prepared in distilled water and copper charged water respectively. The flasks were incubated at RT on a shaker at 120 rpm and absorbance was measured at 600 nm at regular intervals for 48 hr. the experiment was performed at replicates and average readings were plotted to obtain the growth curve.

Results

Copper charged water

For the vessel, surface to volume ratio was measured and found to be approximately 1:1. Copper elution rate for the aliquots using ICP-AES is shown graphically in Figure 1. The water kept for 24hr in the vessel was found to be containing approximately 1ppm of copper ions. pH of this sample was found to slightly decreased from pH-7.3 to pH-7.1. On the other hand conductivity increased from 39 uS/cm to 40.7 uS/cm.

Effect of metals

After exposing the *Candida* culture to different metallic surfaces for 24 hr, copper was found to be the most effective in killing the same, followed by brass, iron, aluminium and steel (Table 1).

Time-point killing assay

The average result of the replicates for this assay after each time interval is given in Table 2. Copper vessel is found to be showing the most potent anti-candida effect followed by copper charged water and then by fluconazole at its Minimum Inhibitory Concentration.

Growth kinetics

Growth curve for both media obtained by plotting Time in hour on X-axis Versus Absorbance at 600 nm on Y-axis is shown in Figure 2. *Candida* grown in media containing copper ions shows an extended lag phase compared to the control. Also the cell density remains less even when the culture entered stationary phase. Copper ions are hampering normal growth of *Candida* compared to the control media.

Discussions

The experiment supports the use of copper vessels as well as *Tamarajal* (copper charged water) as an antifungal agent. The dissolution of copper in the form of its ion gradually increases with time. We can observe the slight metallic sheen develops on the surface of water. The pH decreases slightly as the positively charged ions increases and hence the conductivity also increased slightly. Though intake of 1 ppm copper charged water is not harmful for human, it has power to kill *Candida*.

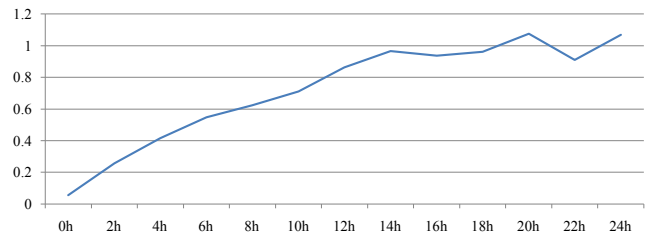

Figure 1: Copper elution graph.

Metals	Control	Steel	Aluminium	Iron	Brass	Copper
CFU/ml	992	934	828	654	370	120

Table 1: Effect of metals on *Candida albicans*.

Time	PBS (control)	Fluconazole (32 ppm)	Copper charged water (1 ppm)	Copper vessel
0 min	1337	1289	1297	1203
60 min	1321	1285	1245	158
120 min	1237	1248	1154	150
180 min	1219	1215	1110	8
24 hrs	1023	798	234	3

Table 2: Time-point killing assay.

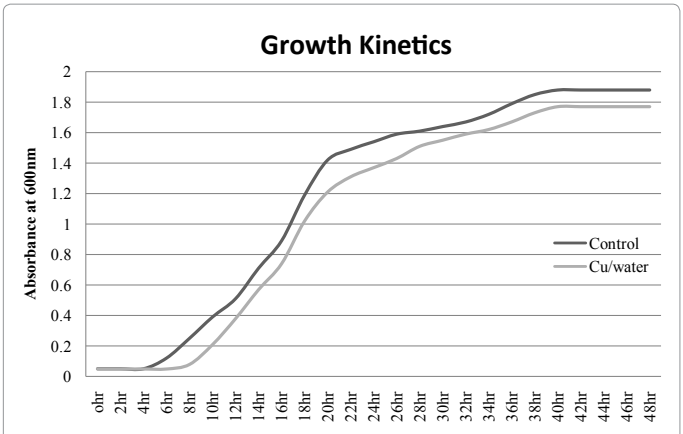

Figure 2: Growth kinetics of *Candida albicans*.

Copper metal is most effective in killing *Candida* while other metals did not show much effect on the same, although brass shows some, only because it is an alloy of copper again.

In order to find out the time required to kill *Candida*, time point killing assay was performed. Here, culture suspension was made in demineralized water to eliminate any reaction between chloride ion in saline and copper ions. Fluconazole was also used to compare its efficacy with metallic copper and copper charged water. As the MIC of fluconazole for *Candida albicans* NCIM 3471 was found to be reported as 32 ppm, this concentration was used in this assay for comparison. Unlike bacteria which are mostly killed within 2 hrs of exposure to copper, *Candida* needs more exposure time for the same.

The copper surface of the vessel was effective within 3hrs on *Candida* whereas copper charged water took 24hrs to be effective. This is because metallic surface exposure by contact is much more toxic than its ions present in water. However, compared to fluconazole which is used as a standard antifungal drug, copper charged water was found to be more potent. The enhanced time of exposure for its activity may be due to the complex organization of *Candida* than bacteria and its ability to uptake copper ions in the cell during initial period of exposure. When growth of *Candida* in its media prepared in copper charged water was studied, the graph indicated an extended lag phase from 4 hrs (control) to 6hr (copper-charged media). This might be due to harsh environment provided by the copper ions in the media. Although the culture gains its exponential phase due to the nutrient availability, its cell density remains less than the control media even in stationary phase.

Future perspectives

Investigations are being carried out on the effect of copper and its water on other fungus as well as its mechanism of action. It can be a boon for developing potent but harmless drugs for the treatment of fungal diseases.

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