



Biocidal action of metal (Mg/Cu/Ni/Zn) doped strontium formate dihydrate crystal against bacterial and fungal strains

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ABSTRACT

Strontium formate dihydrate crystal was grown and Mg, Cu, Ni and Zn metal were doped into the newly grown strontium formate crystal. The pure and metal doped crystals were characterized by EDX, FT-IR and XRD spectroscopic methods. Their antimicrobial activities have been investigated against the series of bacterial and fungal strains. The biocidal activity of pure and doped strontium formate crystals was compared. The effects of dopant concentration on the biocidal activity of the crystals have been also reported. Increase in dopant concentration inside the crystal lattice was resulting in the increase of antimicrobial activity of the crystals.

Keywords: strontium formate; dopant; zone of inhibition; biocidal activity; microbial cell.

INTRODUCTION

Though many attempts and achievements are being made on the design and development of new drugs against the major problem causing bacteria and fungi, infectious diseases caused by them are still a major threat to public health. For example, bacteria related food poisoning is the most significant one for which the food processors, food safety researchers, and regulatory agencies have been increasingly concerned with the growing number of food-borne illness outbreaks [1, 2]. Importantly, nearly 90 percent food poisoning cases for each year are caused by the harmful bacteria such as *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and entero-pathogenic *Escherichia coli*. Emerging resistance of these species is seriously decreasing the number of effective antibacterials. At the mean time, the food industries are forced to reduce the use of chemical preservatives due to increasing pressure of consumers or legal authorities [3]. Not only bacteria, fungi are also very versatile organisms that live in and on animals as part of their natural flora and they can be the cause of numerous infections. Fungal infection is a growing problem in contemporary medicine. The emergences of new diseases like Acquired Immuno Deficiency Syndrome (AIDS), and the re-emergences of old ones, like Tuberculosis (T.B.), have led to an increase in the incidence of fungal infections. Fungi are resistant to the current prescription drugs, so their emergence is a matter of urgent concern [4, 5].

Alongwith the above mentioned problems, the appearance of undesirable side effects of certain antibiotics [6] also lead to the search of new antimicrobial agents. Over the few decades, numerous methods have been adopted for the development of antifungal and antibacterial agents. Often reported methods are extraction of oil from medicinal plants [7, 8], synthesis of new azo class organic compounds [9], sulphur containing organic compounds [10, 11], Schiff base and its complexes [12, 13], silver compounds [14, 15] and transition metal complexes [16-18]. But there is no work reported on the antimicrobial activities of pure and metal doped strontium crystals. With an interest to develop the new drugs against the bacterial and fungal pathogens, here we present the growth and investigation of

antimicrobial activity of the pure strontium formate crystals. In addition, the effect of various dopants on the biocidal action of strontium formate crystal has been also reported.

MATERIALS AND METHODS

The materials used for the growth of sample crystals were strontium carbonate, formic acid (85%), sulphates of magnesium, zinc, nickel and copper and double distilled water. All the chemicals used were AnalaR grade supplied by Ranbaxy and CDH.

2.1. Synthesis of strontium formate dihydrate

18.5 ml of 85% formic acid was mixed with 150 ml of doubly distilled water. The solution thus obtained was kept in a constant temperature bath at 40 °C for half an hour to warm the solution. To this warmed solution, 29.5 gm of strontium carbonate is added in small portions so that foaming is not excessive. The solution is stirred continuously in a magnetic stirrer till all the carbonate has dissolved and bubbles of carbon dioxide is no longer formed in the solution. The solution thus obtained is filtered and transferred to 100 ml beakers and sealed. The sealed beaker containing the solution is kept in a constant temperature bath at 35 °C and allowed to evaporate slowly. Strontium formate dihydrate crystals were formed in two to three days time. These crystals were collected, stored and used as starting materials for the growth of pure and doped strontium formate dihydrate crystals in the present study.

2.2. Growth of pure and doped strontium formate dihydrate crystals

The saturated solution of strontium formate dihydrate was kept in a magnetic stirrer at a temperature of 40 °C for a period of 2 h. The solution thus obtained was filtered and transferred to the growth vessel (100 ml beaker) and kept in a constant temperature bath with an accuracy of ± 0.1 °C. The temperature of the bath was maintained at 40 °C throughout the growth process. For the growth of doped crystals the sulphates of the respective dopants (magnesium, zinc, nickel and copper) were added to the saturated solution in the fixed dopant concentration. The dopant concentrations fixed in the present study were 0.005 and 0.05 M.

2.3. Biological active tests

2.3.1. Microbial strains

The tested microorganisms were clinical, animal and food isolates with known resistance pattern to common practice antimicrobial factors were provided from the Department of Microbiology, VHNSN College, Virudhunagar, India. Two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative (*Pseudomonas putida* and *Escherichia coli*) bacterial species were selected for the investigation of antibacterial activity of the crystals. The selected fungal strains for the study of antifungal activity of the crystals were *Aspergillus niger*, *Fusarium solani*, *Curvularia lunata* and *Candida albicans*. The isolates were biochemically and serologically characterized by standard methods [19]. These eight species are very common pathogens causing a variety of human and animal infections and well recognized for resistance to a number of antimicrobials used in the medical and veterinary practice. Their cultures are of low cost and easily maintained and they are supposed to be typical representatives of Gram+ve /Gram-ve bacteria and fungi.

2.3.2. Culture media and inoculum

The strains of bacteria were preserved on nutrient broth, while the strains of fungal species were maintained on Sabouraud dextrose agar. A well was made on the agar medium inoculated with microorganisms and was filled with the test solution using a micropipette and the plate was incubated at 37 °C for bacteria (24 h) and 25 °C for fungi (48 h). During this period, the test solution diffused in the well and the growth of the inoculated microorganisms was affected. The inhibition zone was developed around the well and measured in mm. Chloramphenicol and Amphotericin were used as control drugs for bacteria and fungi, respectively.

2.3.3. Antimicrobial assay

The antimicrobial effect of the pure and doped strontium dihydrate crystals were tested using the agar well diffusion method following the well-established method of Deans and Ritchie [20]. Overnight bacterial cultures were used for surface inoculation of Petri dishes containing 15 mL of nutrient broth. Each Petri dish was spread on with 0.5 mL of strain inocula streaked thoroughly all over the surface of the nutrient broth. Subsequently, four equidistant wells, 4 mm in diameter each, were punched into the inoculated medium with sterile glass Pasteur pipettes and were filled up with test solution using a precise pipettor. All plates were incubated at 37 °C and inhibition zones were measured after 24 h. All the experiments were repeated twice, including two controls with plain DMSO and sterilized distilled water every time. After incubation, the inhibition zones were measured to an accuracy of 1 mm and the effect was calculated as a mean of the duplicate experiments for each triplicate strain test.

RESULTS AND DISCUSSION

3.1. FT-IR spectroscopy

The observed bands from FT-IR spectra of pure and doped crystals were confirmed the formation of pure and doped strontium formate dihydrate crystals. The observed bands for pure and Mg doped strontium formate is given below: Pure strontium formate, ν (cm^{-1}): 3661 & 3416 (H-O-H), 2984 (C-H), 1589 (C-O), 1252 (C-H bending) and 664 & 589 (Mg-O). Magnesium doped strontium formate: 3658 (H-O-H), 2980 (C-H), 1582 (C-O), 1245 (C-H bending) and 662, 590 & 565 (Mg-O).

3.2. EDX analysis

Figure 1a and 1b show the EDX spectrum for pure and 0.05 M magnesium doped strontium formate dihydrate crystals, respectively. The spectrum confirms the presence of magnesium in the crystal along with Sr, C and O atoms. The results show that there is reasonable agreement in the concentration of magnesium ions in the grown crystals with that of actually taken for experiment. Introduction of Mg^{2+} ion into strontium formate crystal may lead to the formation of Mg^{2+} HCOO^- dipoles (Mg^{2+} substitutes Sr^{2+}). These results are in accordance with the results reported earlier for other Mg-doped metal formate crystals [21].

3.3. XRD studies

The lattice parameters for pure strontium formate dihydrate crystals calculated in the present study were, $a = 6.853(27)$ Å, $b = 8.744(14)$ Å and $c = 7.259(21)$ Å. The values reported in the literature were $a = 6.864(1)$ Å, $b = 8.752(1)$ Å and $c = 7.262(1)$ Å [22] and the lattice constant increases with dopant concentration. This further confirmed that the dopant has entered the crystal lattice. The lattice parameters obtained in the present study are in good agreement with the values reported in the literature for doped crystals [24].

3.4. Antimicrobial studies

Antimicrobial studies on metal doped and pure strontium formate dihydrate crystals have been done by well diffusion method. It is noted that metal doped and pure crystals were able to destroy the colony growth of microbes to some extent. The antimicrobial ability of the crystal has been calculated from the zone of inhibition values in mm, which encircles the crystal solution. Greater the zone of inhibition value, greater will be the biological activity.

3.4.1. Antibacterial activity

The antibacterial activity of Mg, Cu, Ni and Zn doped strontium formate dihydrate crystals is calculated by their zone of inhibition values and they are compared in the figures 2a-2d, respectively with pure strontium formate and commercial drug. From the figures 2a-2d, it is observed that pure strontium crystal shows comparable antibacterial activity with the commercial standard antibacterial agent, chloramphenicol. But, after inclusion of dopant into strontium formate dihydrate crystal matrix, the antibacterial activity of the crystal gets significantly increased. The zone of inhibition values are greater at 0.05 M dopant concentration in strontium formate crystal than 0.005 M metal doped crystal solutions. It shows the significance of the dopant concentration on the antibacterial activity of the strontium formate crystal. In addition, the doped strontium formate crystal test solutions exhibit better antibacterial activity than even the commercial drug, chloramphenicol. This better catalytic activity of doped crystals is attributed to the more metal content in the doped strontium formate crystal solution. The previous reports also proved that increase in metal content of the drug increases the antibacterial activity of the drugs [24, 25]. The reason for this increase in antibacterial activity of doped strontium formate crystal may be due to the increase in stability with high inorganic content (metal) after dopant addition. Generally, the stability of inorganic material based drugs is higher than that of the organic material based drugs [24-26]. So, the doping of metal to strontium formate crystal further increases the inorganic content in the crystal and it leads to the increase in the biocidal activity against bacteria. This increase in stability after dopant addition provides the long life time for the crystal to act against the bacterial strains. Among the reported bacterial strains, the strontium formate crystals strongly act against *S. aureus* and *E. coli* as reported in the literature [27]. But all the crystals showed lowest antibacterial ability against *P. aeruginosa* because of its relatively impermeable membrane, constitutively expressed and inducible efflux systems and a chromosomally encoded inducible β -lactamase. It is in well accordance with the previous report [26]. Among the various doped crystals, magnesium doped crystal shows high antibacterial activity. It may be attributed to the difference in the metallic properties of magnesium and other metals (Cu, Ni and Zn). The smallest ionic radius of Mg(II) ion which enhances its diffusion inside the microbial cell is also the reason for the ceiling antibacterial activity of magnesium doped crystals.

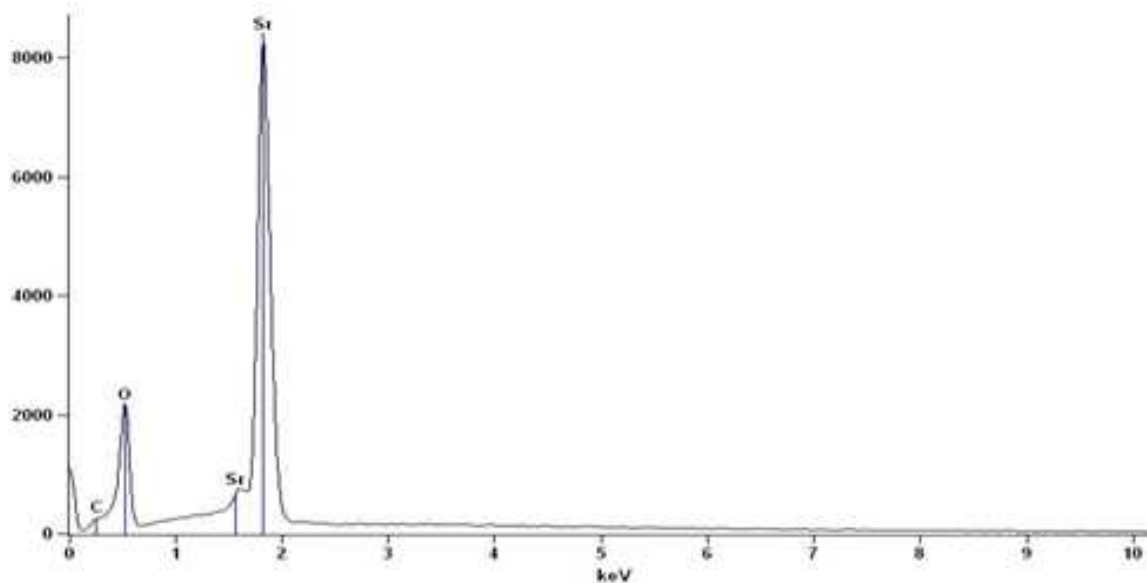


Figure 1a. EDX spectrum of pure strontium formate dihydrate crystal.

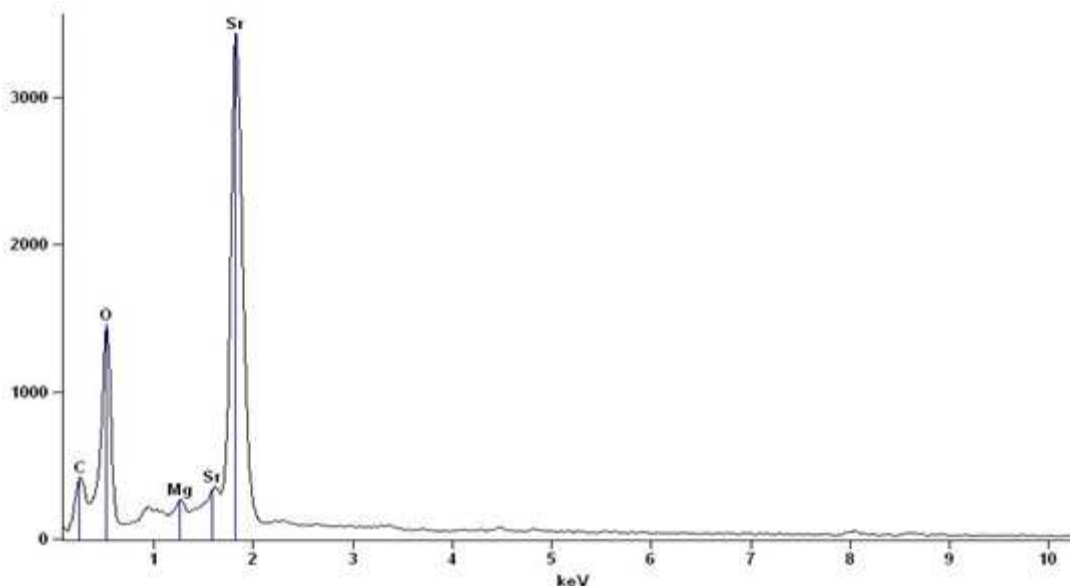


Figure 1b. EDX spectrum of 0.05 M Mg doped strontium formate dihydrate crystal.

3.4.2. Antifungal activity

Figures 3a-3d show the antifungal activity of Mg, Cu, Ni, and Zn doped crystals, respectively against the series of fungal spectrum by comparison with the reference standard, amphotericin. Like the antibacterial activity, the antifungal activity of the pure strontium formate crystal is well comparable with the commercial drug. Effect of dopant concentration on antifungal activity of strontium formate crystal becomes the remarkable one, since the increase in dopant concentration greatly increases the antifungal activity of the pure crystal. This increase is allocated to the increase in stability of the doped crystals inside the cell of the fungal strains. The doped crystals exceed the biological activity of the commercial drug, Amphotericin. Pure and metal doped crystals show highest antifungal activity against *C. albicans*. The previous reports also reveal that the chemical based drugs exhibit better biocidal activity against *C. albicans* than other fungal strains [28, 29]. The magnesium dopant shows highest impact in the antifungal activity of strontium formate dihydrate crystals over all other metal doped crystals. This may be due to the smallest ionic radius of Mg(II) ion which facilitates the faster diffusion through the cell membrane and the strongest biological and powerful chemical interaction of alkali earth metals over the transition metals.

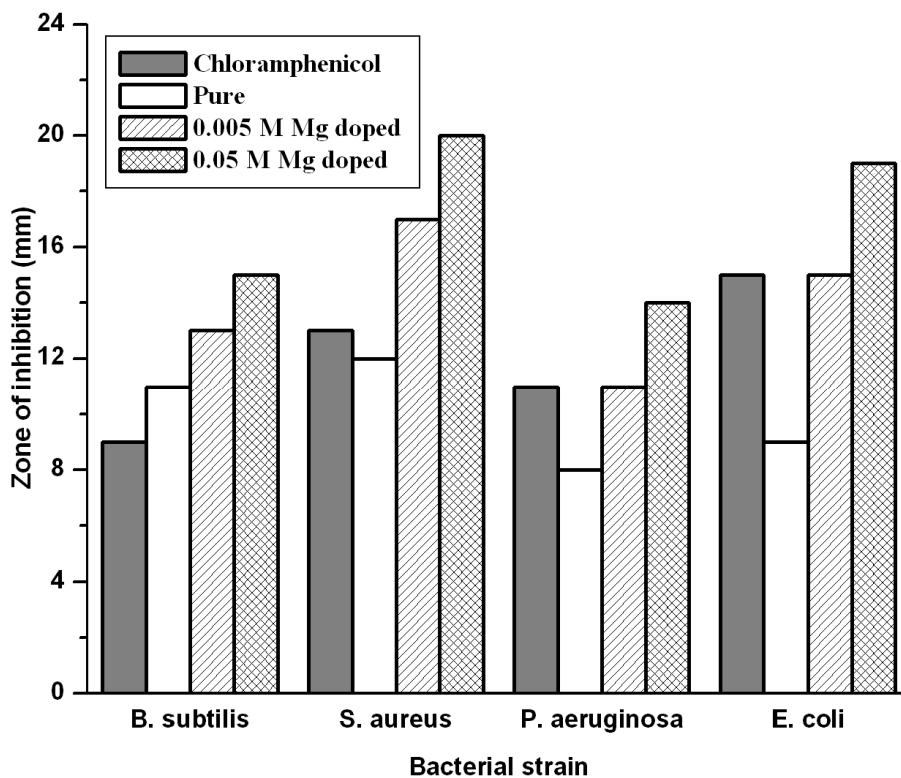


Figure 2a. Antibacterial activity of Mg doped strontium formate dihydrate crystal.

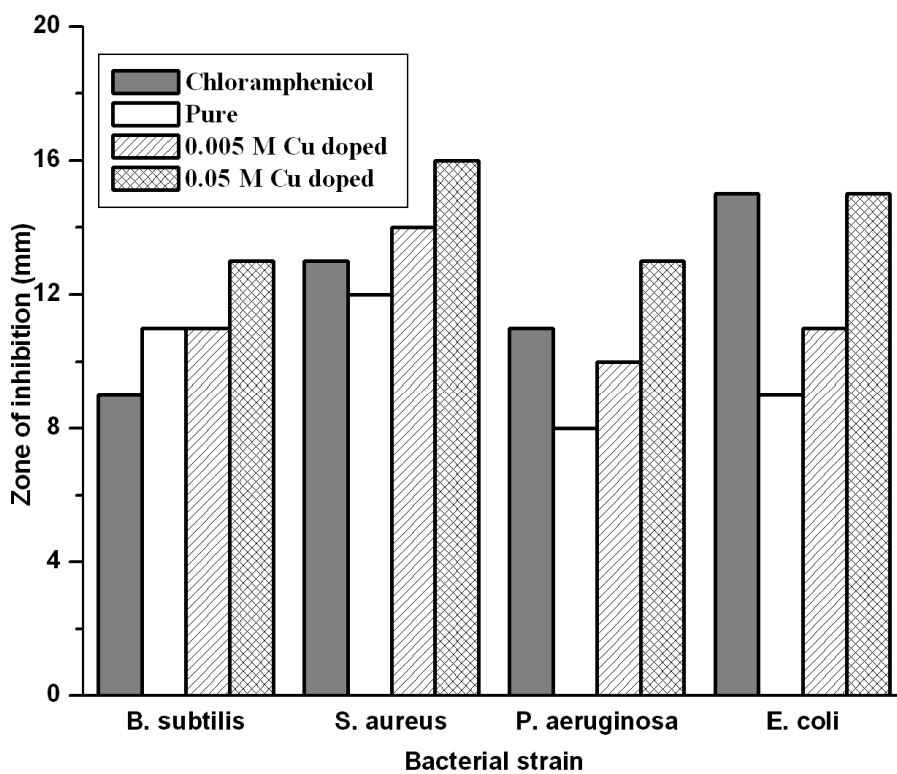


Figure 2b. Antibacterial activity of Cu doped strontium formate dihydrate crystal.

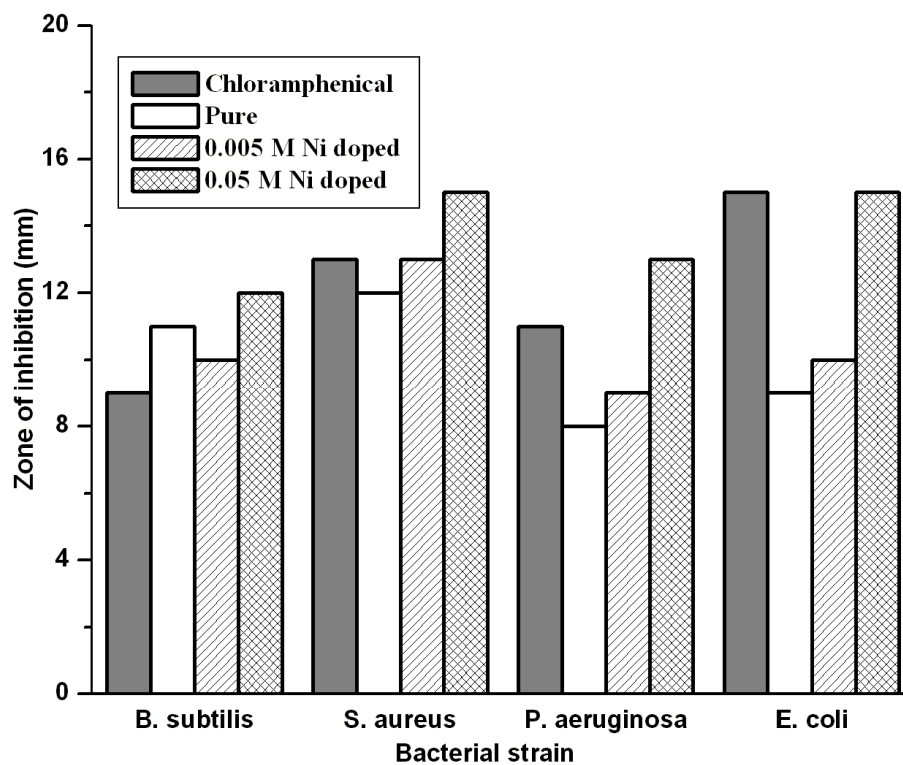


Figure 2c. Antibacterial activity of Ni doped strontium formate dihydrate crystal.

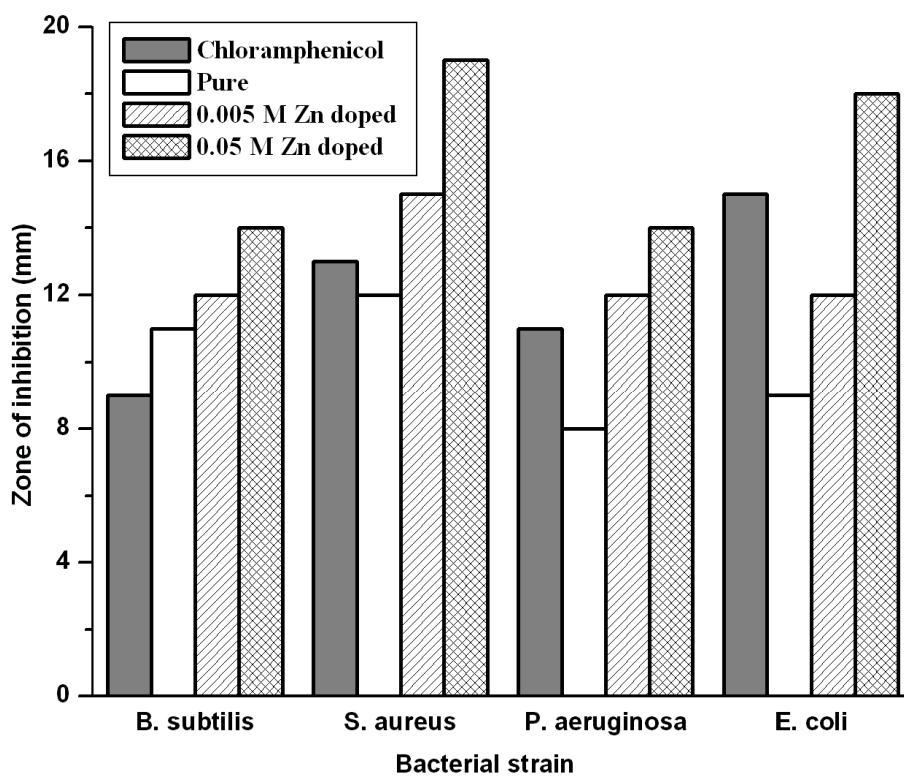


Figure 2d. Antibacterial activity of Zn doped strontium formate dihydrate crystal.

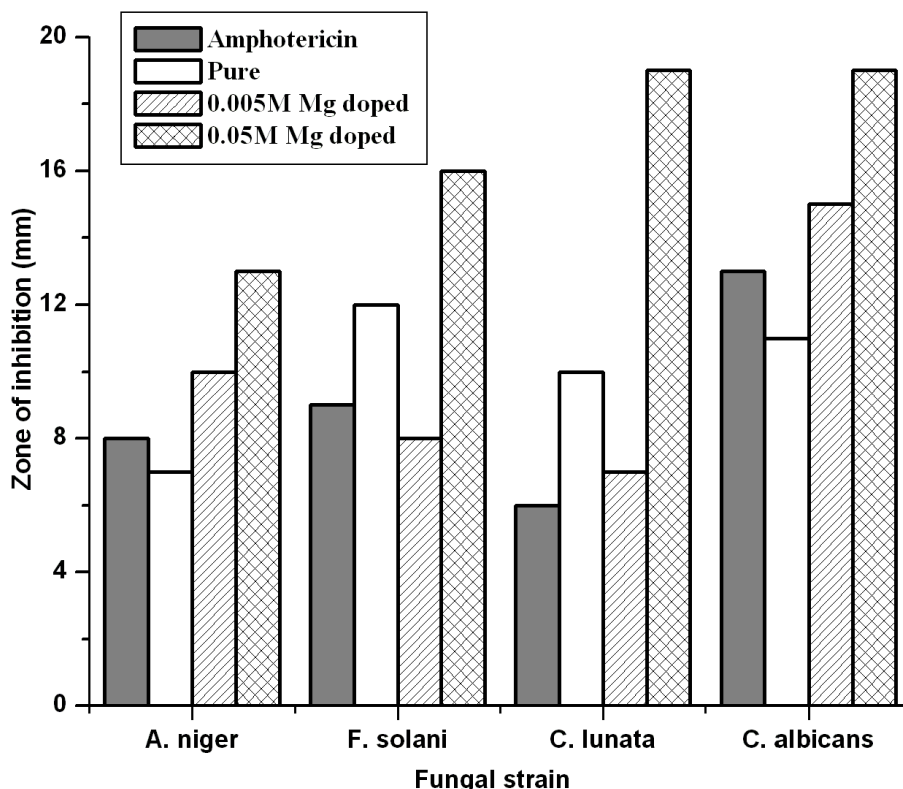


Figure 3a. Antifungal activity of Mg doped strontium formate dihydrate crystal.

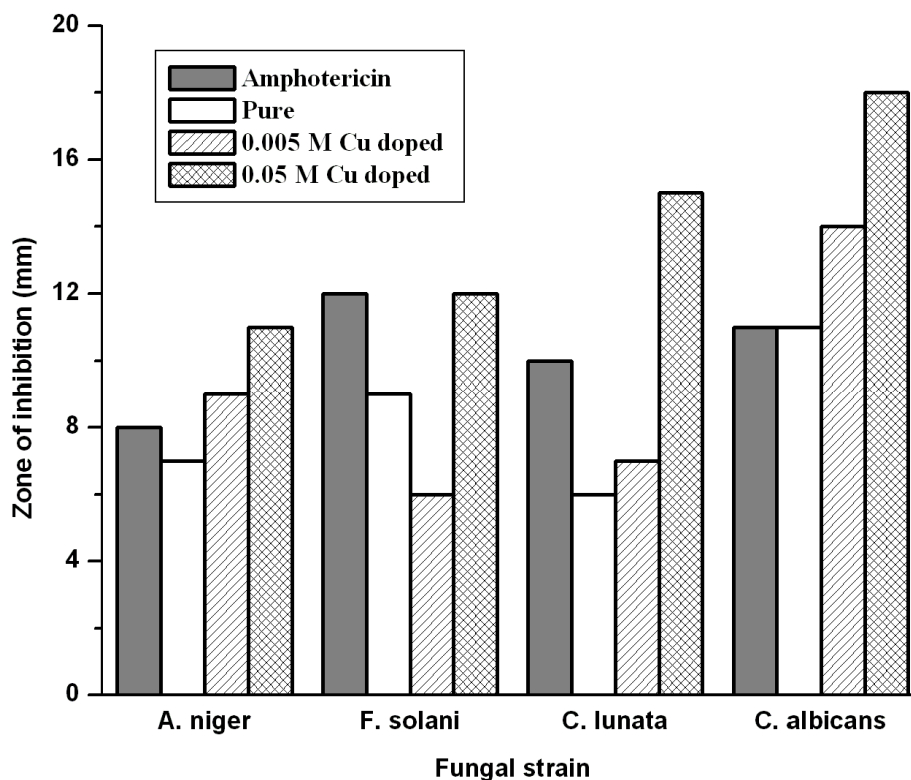


Figure 3b. Antifungal activity of Cu doped strontium formate dihydrate crystal.

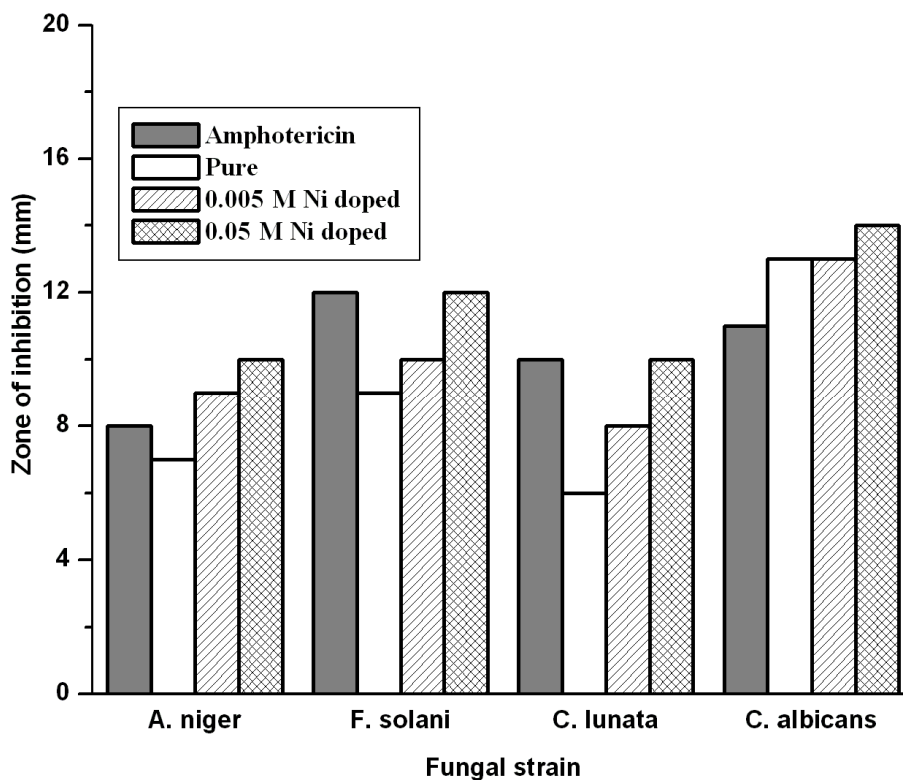


Figure 3c. Antifungal activity of Ni doped strontium formate dihydrate crystal.

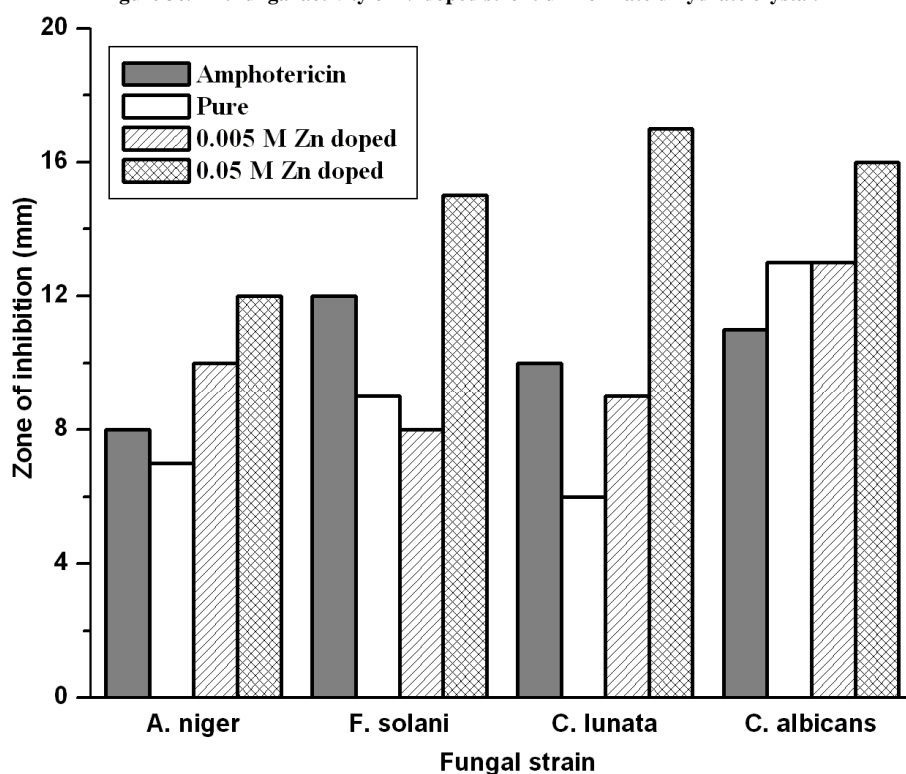


Figure 3d. Antifungal activity of Zn doped strontium formate dihydrate crystal.

3.4.3. Mechanism for biocidal action of crystals

Many reasons have been suggested for the antimicrobial activity of the chemical based drugs, but still it is not clear. The possible mechanisms for the biological action of pure and doped strontium formate crystals are listed below:

1. Increase in lipophilicity upon the addition of crystal with microbes near the cell membrane is the strongest proposed reason for the inhibitory activity of the crystals [29-31].

2. It is also believed that metal ions enter through the cell membrane, causing massive leakage of intracellular substances and eventually causing cell death.
3. Metal ions in the crystal solution, attach to phosphate and sulphur groups that are part of the phospholipid cell membrane or to membrane proteins and severely damage the cell and its major functions, such as permeability, regulation of enzymatic signalling activity and cellular oxidation and respiratory processes [32, 33].
4. Metal ion in the crystal can also penetrate the microbial cell, bind to the DNA inside the bacterial cell, hence preventing its replication.
5. The biocidal action of crystal solution is also possible because of mechanistic pathways of metal cation with the microbial cells depend upon the site of action. It may be due to non-specific reaction outside the cell protoplast or may be due to the formation of un-ionized complexes with electron dense area of the microbial cells.

CONCLUSION

The biocidal activity of pure and metal doped strontium dormate crystals have investigated successfully by well diffusion method. Crystals showed the better antibacterial activity against *S. aureus* and *E. coli* than other bacterial strains. The growth of *C. albicans* was well inhibited by the crystals compared to other fungal strains. This study on the effect of dopant concentration on the biocidal activity of strontium formate crystal has concluded that the biocidal activity has increased upon the increment of dopant concentration. Mg doped crystals revealed good biocidal activity than other doped crystals due to its small ionic radius which enhances its diffusion inside the microbial cells.

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