



ANTIBACTERIAL ACTIVITY OF SILICEA, CALC CARB, SULPHUR ON GRAM NEGATIVE BACTERIA E.COLI

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ABSTRACT

Increase in use of antibiotics in recent days have resulted in development of drug-resistant bacteria, which ultimately can be life threatening. As an alternative solution to this problem, we have used homeopathic medicines for our study of antibacterial activity of E.coli, a gram negative bacteria. Activities of Silicea, Sulphur and Calcarea carb (Calc carb) at the potency 6 C, 30 C, and 200 C were observed using general spread plate techniques. For different potencies of each of these drugs, activities are different. Among these three drugs sulphur is found to be more active. At 200 C potency silicea and Calc carb had their minimum colonies, whereas for sulphur at 30 C potency growth is inhibited.

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INTRODUCTION

Human body contains more bacterial cells than human cells, which are essential for overall health. The food we eat contains many different bacteria, from various sources, some of which are harmful and a large number of people get sick yearly from these harmful bacteria. This food passes through the gastrointestinal system, where the colon is lined with millions of bacteria that help prevent the growth of harmful bacteria, maintain the mucosal immune system, help lower serum cholesterol levels and regulate metabolism.

The well known gram negative bacteria *Escherichia coli* (*E.coli*) cover a large part of the intestine. Its harmless strains provide vitamin K₂ and prevent colonization of other harmful bacteria of the intestine. However, some strands of *E.coli*, can be pathogenic, leading to diarrhea, urinary tract infection and intestinal illnesses, which can be cured by using suitable antibiotics. Some of these antibiotics have serious side effects, while their uncontrolled use leads to development of drug resistance bacteria.

We report here how the infection caused by *E.coli* can be treated by using homeopathic medicine, which has insignificant side effects and associated complications. For microbial study of effect of homeopathic medicine on *E.coli*,

we have used three medicines, namely Silicea, Calc. Carb and Sulphur at 6C, 30C and 200C potency.

MATERIALS AND METHODS

To find the particle size of these homeopathic drugs at different potency we used Scanning Electron Microscope (Inspect FEI, F50 Netherlands). The three medicines Silicea, Calc carb and Sulphur at three different potencies 6C, 30C and 200C, prepared as per standard procedure [Homeopathic Pharmacopoeia of India, 1971, published by Ministry of Health, Govt. of India. The Homeopathic Pharmacopoeia of the United States (on-line version)] were obtained from HAPCO, India. The bacterial strain of *Escherichia coli* DH5 alpha (MTCC no.1652) used was obtained from Institute of Microbial Technology, Chandigarh, India. All the chemicals were used without further purification. Throughout the experiment double distilled water was used.

Preparation of culture- Fresh bacterial strains were grown in recommended nutrient broth which contains peptone (0.5%) beef extract (0.1%), yeast extract (0.2%), NaCl (0.5%) and distilled water and kept overnight. Next day, *E.coli* was treated individually with the three medicines at three potencies (6C, 30C, 200C) and kept overnight. Agar plates were prepared by

A) SEM IMAGE OF SILICEA AND SULPHUR

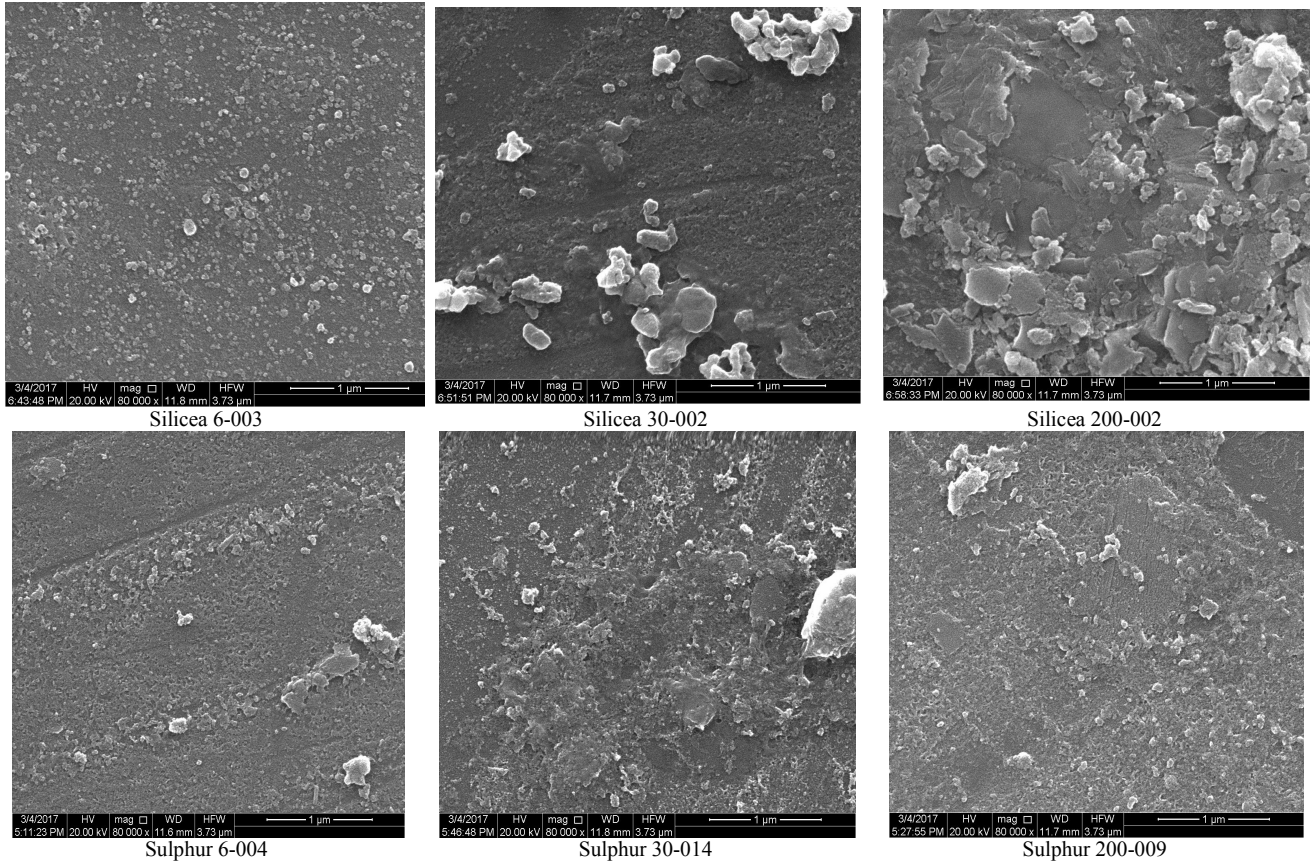


Figure 1 scanning electron microscopic image of silicea and sulphur.

It is clearly evident from the SEM images that except for Silicea, the particle sizes decrease with increasing potency. In silicea, main component is silica. According to the silica theory, with increasing potency, when the amount of succussion is high, it helps in leaching silica from the container wall. This is observed in the SEM image. Sulphur, is non-metallic in origin, which is evident from the SEM image.

B) growth inhibition of *e.coli*

Growth of bacterial colonies in presence of three different drugs at three different potencies are shown in Figures 2 to 4 and compared with the control i.e., in absence of the drug.

Figure 2 antibacterial activity of different potencies of silicea against *E.coli*.

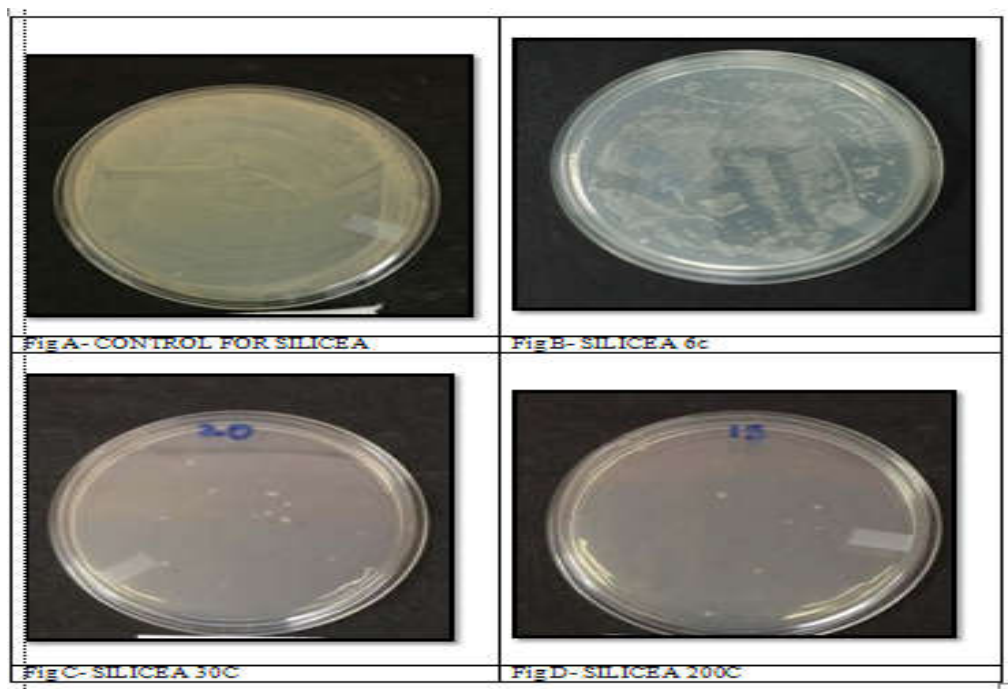


Fig. 2 Image A) – Control, that shows the uninhibited growth of bacteria without any drug. Image B), C), D) plates were treated with the drug silicea at 6C, 30C, 200C potencies respectively.

Figure 3 antibacterial activity of different potencies of calc carb against *E.coli*.

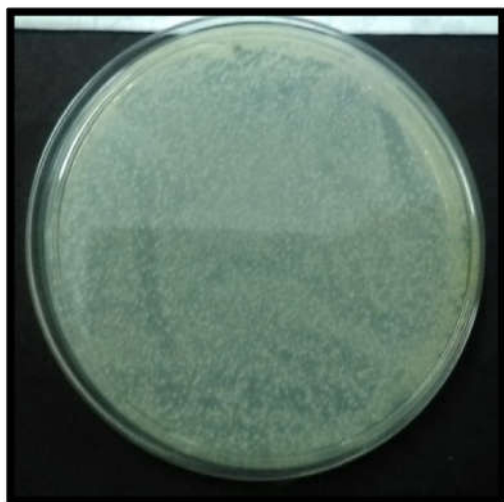


Fig A-CONTROL FOR SULPHUR



Fig B-SULPHUR 6C



Fig C- SULPHUR 30C



Fig D- SULPHUR 200C

Fig. 4 Image A) – Control, that shows the uninhibited growth of bacteria without any drug.

Image B), C), D) plates were treated with the drug sulphur at 6C, 30C, 200C potencies respectively. The images indicate that bacterial growth gets restricted with the drug even at 30 C

Fig 2 indicates that the bacterial growth is minimum in case of the colonies treated with the drug Silicea at 200C. Similar results were observed in case of Calc carb (Fig. 3). But in case of Sulphur it was observed that bacterial growth gets restricted to a large extent even with drug at potency 30 C (Fig. 3).

adding 1.8% agar-agar. 50µl of sub cultured bacterial strain were spread in each agar plate and colonies were counted with reference to control plate

RESULTS

Scanning Electron Microscopic Study of three homeopathic medicines at three potencies

The morphology of the following 6 homeopathic medicines at the potencies of 6C, 30C and 200C were studied by Scanning Electron Microscopy.

- Calcarea carb: Carbonate of lime (Image not reproducible and hence not shown).
- Silicea: Silicon dioxide SiO₂.
- Sulphur: Sulphur atoms form cyclic octaomic molecule S₈.

The SEM pictures of these medicines were taken several times and the results at magnification of 80,000 are presented here.

DISCUSSION

From the Scanning electron microscope studies we observe that potentization, changes the mean size of nanoparticles of the homeopathic drugs. The above experiments clearly show that the drugs Silicea, Calc carb and Sulphur work as potent inhibitor of growth of *E.coli*. The result indicates that compared to the control where no drugs were used for treatment, out of the three different potencies namely, 6C, 30C and 200C of the three drugs used; the antibacterial activity is higher for higher potency of the drug. The drug at potency 200C showed maximum antibacterial effect by killing the maximum number of bacteria and only few numbers of colonies were left on the agar plate, whereas drug at 6C has minimum effect. At higher potency of homeopathic drug, the bacteria are unable to grow (Ghosh B et al 2017) We have observed that sulphur is quite effective even at 30C potency. This is because it has been experimentally proved that there is antimicrobial activity of sulphur on human pathogenic organisms and its activity depends upon the size, being more for smaller particles (Weld J.T., Gunther A. 1947). Thus in

case of sulphur, the inhibitory effect becomes quite dominant even at lower potency.

Drug particles are attached to the membrane of bacterial cell by electrostatic interaction and disrupt the integrity of the membrane of the cell. With succussion, mechanical energy is transferred and breaks the drug aggregates to form smaller particles (Shah R 2016). Thus at higher potency, i.e. at high dilution associated with high succussion, the individual particles are of nanodimension, (Kar S et al 2015) and can better penetration through the membrane barrier (Nandy P et al 2011). They also have less chance to aggregate and so each individual particle can have its own separate surface area to interact with the bacterial membrane. More the surface area more is the chance of interaction which leads to more toxicity. This explains why higher potency of nanomedicine showed more antibacterial activity and destroys bacterial cells.

CONCLUSION

From the above experiments it can be concluded that the three homeopathic drugs Silicea, Calc carb and Sulphur do have inhibitory effect on the growth of *E. coli* colony. For the first two drugs, the maximum inhibitory effect on the growth of *E. coli* bacteria was obtained at 200 C potency, while sulphur having a size dependent antimicrobial effect, can inhibit the growth even at 30C.

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