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Screening of Minimal Inhibitory & Bactericidal concentration (MIC & MBC) of purified Thurusu

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Abstract

Crude copper sulphate(CuSo4) or *thurusu* is used as a raw drug in Siddha system of Medicine, it is available in nature and prepared synthetically by boiling copper with sulfuric acid. Chemically it exists in two forms hydrated form copper sulphate pentahydrate, it is blue in colour and anhydrous form copper sulphate ,it is pale green or grey white in colour. It is used after purification as a single drug or one of the ingredients in some metallo mineral formulations, it is widely used for external applications and internal formulations it has astringent, emetic, tonic, caustic and antiseptic actions, *thurusu* is normally used to heal ulcers, ulcers in male genital organ, eye diseases, *tiridhodam* etc .

Hence in this pretext the bactericidal concentration of purified *thurusu* is validated by Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) methods.The MBC test allows determination of the minimum concentration of an agent necessary to achieve a bactericidal effect. It is worth noting, however, that the duration of time the antimicrobial is in contact with the test organism is quite long for this method, on the order of 18 hours. Thus, the test truly does determine the minimum concentration needed to kill the test organism, since all other parameters are conducive to biocidal effect. The MBC test can be a good and relatively inexpensive tool to rank a great number of antimicrobial agents by potency, for screening purposes. The MBC test can be used to evaluate formulation problems wherein the formulator suspects that the active ingredient is being “bound up” by other ingredients. The theory is that the MBC will be worse for a formula that has a portion of its active ingredient chemically combined with other ingredients, thus not available to kill microorganisms in the suspension.

Keywords: *Thurusu*, MIC and MBC , Pre- clinical studies, Anti-bacterial

1. Introduction

A Siddha medical system is the oldest in holistic management and it was being practiced by a large population in South India. Traditional system of medicines were used by 60% of the world's population for their health care in developing and developed countries even though where modern medicines dominate. Thus this of medicine was a boon offered by the spiritual scientist called Siddhars. There are so many Siddhars, out of them eighteen Siddhars are the most important and they all have more knowledge about the universe and its contents. Siddhars believes that, there is a connection between the celestial bodies of our universe to the living beings of earth. Any changes in the external world, brings changes to human beings. So they believe a healthy body is essential to attain eternal life.

The fundamental principles of Siddha science is 96 principles, Three humors (*Vadha, Pittha, Kapha),"Panchaboothas"* which advocates curative and preventive measures and educates systemized life style through natural way and gives total perfection for life. According to the Siddha science of medicine, diet and lifestyle plays a major role in maintain health and curing diseases.

Standardization is essential for globalization traditional medical systems. Mortality rate was increased day by day due to severity of diseases but also with the adverse effect of the synthetic drugs. Because of this people from different parts of the World preferred to choose natural products as medicines for their health care remedies. Thus it is the best time to explore siddha medicines to the World with minimum adverse effects, less expensive and easy affordable. Thus attempt was made to standardize the siddha drug through a scientific technique MIC & MBC.

2.Determination of minimal inhibitory concentration

2.1. Materials required:

Nutrient Broth

13g of Nutrient Broth media (HiMedia) was dissolved in 1000ml distilled water and was autoclaved at 121°C; 15lbps for 15 minutes.

Culture of Test organisms (Growth adjusted to 1% McFarland Standard):

Streptococcus mutans (ATCC 25175)

DMSO: Dimethyl Sulphoxide (HiMedia)
96 Well microtiter plate
ELISA plate reader (ERBA, LisaScan)
MBC values are calculated by using **ED 50 PLUS V1.0 Software**

2.2. Methods required:

Minimal inhibitory concentration (MIC) was determined by using two fold serial dilution method.The growth of stock inoculum was adjusted to 1% McFarland Standard. The broth dilution assay was done in 96 well microtiter plate.

Each wells in the plate were added with 100µl of the diluted (two times) conidial inoculum suspensions (final volume in each well, 200 µl).

Sample was dissolved in DMSO to a final concentration of 10mg/mL and was added in increasing concentration such as 62.5µg, 125µg, 250µg, 500µg, 1000µg to the wells respectively and incubated overnight at room temperature.

A control well was kept with organism alone.

Growth was observed by visual inspection and by measuring the optical density (OD) at 630 nm using an ELISA plate reader.

The OD was measured immediately after the visual reading.

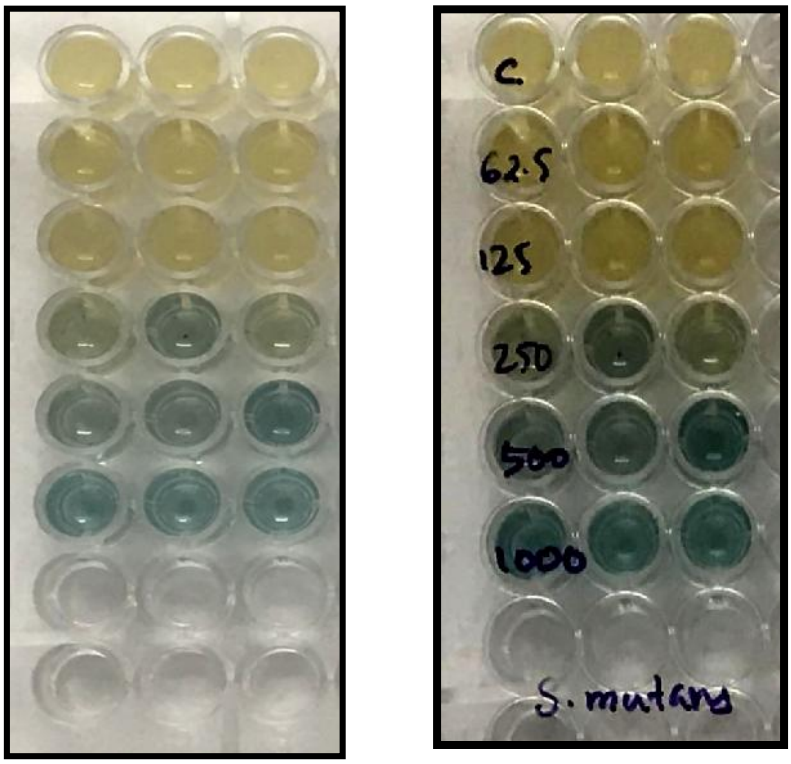
The growth inhibition for the test wells at each extract dilution was determined by the formula:

Percentage of inhibition = (OD of control - OD of test)/ (OD of control) × 100

2.3. Observations of Minimal Inhibitory Concentration

Table.1. Results of Minimal Inhibitory Concentration

Organism: *Streptococcus mutans* (G +)					
Concentration(µg)	**OD 1**	**OD 2**	**OD 3**	**Average**	**% of inhibition**
Control	0.6559	0.6405	0.6385	0.6449	-
Sample Code- A					
62.5	0.4560	0.4538	0.4623	0.4573	29.07
125	0.4334	0.4093	0.4314	0.4247	34.13
250	0.2069	0.2232	0.2351	0.2217	65.61
500	0.1535	0.1617	0.1687	0.1613	74.98
1000	0.1045	0.1010	0.1123	0.1059	83.57



i **ii**

Fig.1. Results of Minimal Inhibitory Concentration of purified Thurusu (Copper Sulphate) against *S.mutans*
i) Control
ii) Sample

2.4. Results of MIC of purified Thurusu (Copper Sulphate) :

By increasing the amount of administrated purified *thurusu*(Copper Sulphate)in 1000 µg concentration, showed that the suppression of *S.mutans* growth in the selected media with 83.57% MIC value.

3. Determination of minimal bactericidal concentration

3.1.Materials required:

Nutrient Broth

13g Nutrient broth was suspended in 1000 ml of distilled water and was autoclaved at121°C; 15 lbs for 15 minutes.

Muller Hinton Agar plates

38g MHA medium (HiMedia) was weighed and was dissolved in 1000 ml distilled water and was autoclaved at 121°C; 15 lbs for 15 minutes. After autoclaving, the media (20ml) was allowed to cool to 60°C and was poured to pre-sterilized petrii;000000000 plates. The plates were allowed to solidify in a laminar air flow chamber.

Bacterial culture:

Streptococcus mutans (ATCC *2*5175) (Growth adjusted to 1% McFardsStandard)

96 Well microtiter plate

MBC values are calculated by using **ED 50 PLUS V1.0 Software**

3.2.Methods required:

The *in vitro* minimum bactericidal activities (MBCs) were determined for each sample against *Streptococcus mutans*. The initial steps were done as in MIC protocol described earlier; (Each wells in the plate were added with 100µl of the two times diluted inoculum suspensions (final volume in each well, 200µl).

Samples were added in increasing concentration such as 125, 250, 500,1000µg to the wells respectively and incubated for 24 hours at room temperature.

A control well was kept with the organism alone.) After 24hours of incubation, 20µl from each well (250, 1000µg) was swabbed on Muller Hinton agar plates; the contents of the wells were not agitated prior to removal of the specified volumes.

The plates were then incubated at 37˚C for 48 hours. After incubation the plates were observed for the presence of colony forming units. The MBC was the lowest drug concentration that showed either no growth or fewer than three colonies to obtain approximately 99 to 99.5% killing activity.

3.3.Observations of Minimal Bactericidal Concentration (MBC)

Organism: *Streptococcus mutans (G +ve*)

Table.2.Observations of Minimal Bactericidal Concentration

Sample	Concentration(µg)	No of colony counted	CFU/mL
	Control	1884	$94.3*10^3$
	250	1312	$65.6*10^3$
	1000	0	-

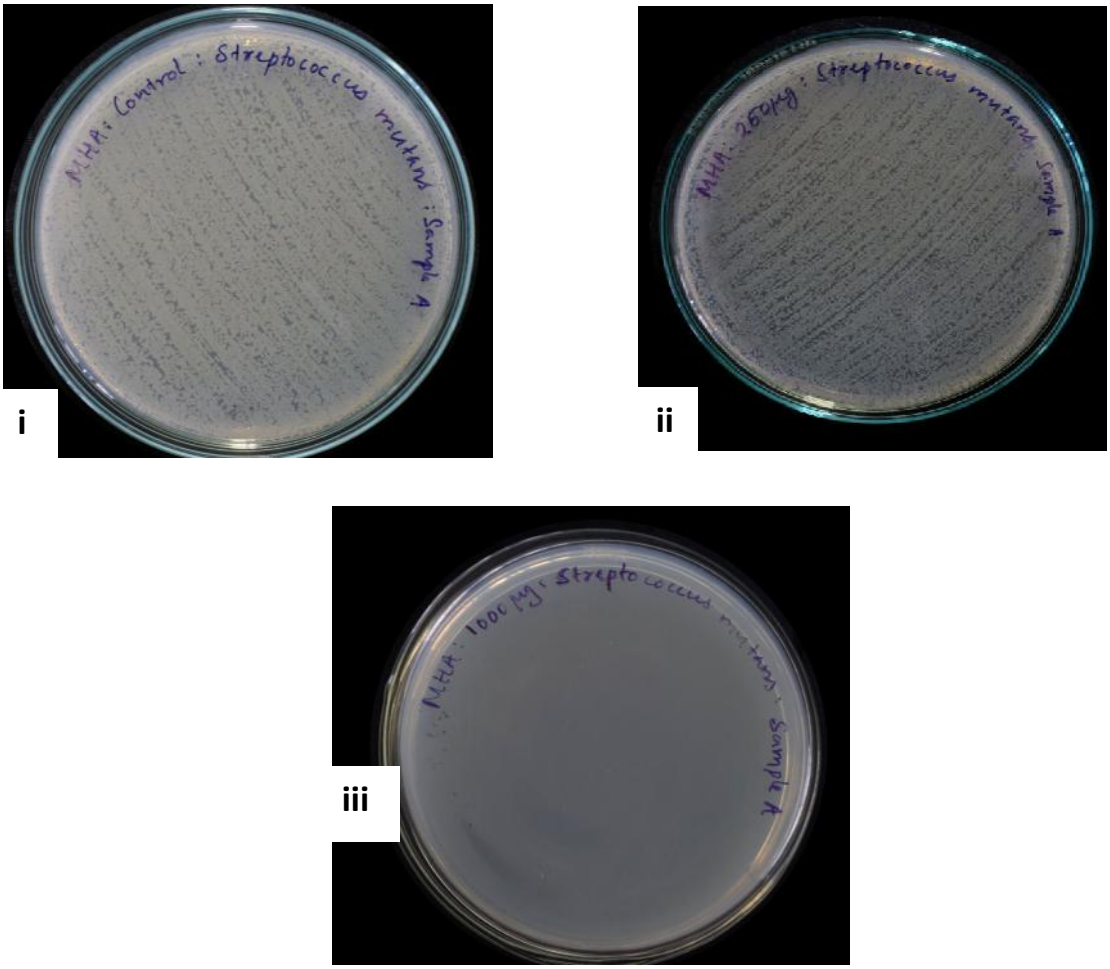


Figure. 2. Observations of Minimal Bactericidal Concentration (MBC)
i) Control ii) Sample 250µg iii) Sample 1000µg

3.4. Results of MBC of purified Thurusu (Copper Sulphate) :

By increasing the administration ofpurified *thurusu* (Copper Sulphate)in 1000µg concentration, showed that the disappearance of *S.mutans* colonies in the selected media

Results and Discussion

The Minimal Inhibitory Concentration and Minimal Bactericidal Concentration of purified thurusu against the gram positive organism *Streptococcus mutans* were significantly noted.

Conclusion

This study shows that *thurusu* (copper sulphate) inhibits *Streptococcus mutans*multiplication in the concentration of 1000µginhibited almost all the bacterial colonies. However, further studies are needed to assess the microbial response to *thurusu* (copper sulphate) *in vitro and vivo*. The biological variability of individual bacterial cells and strains needs to be identified to optimize the *thurusu* (copper sulphate) concentration required to inhibit bacterial growth. The results of this study will be useful for developing a quantitative microbial risk assessment.

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