

# An Invitro Study on the Effectiveness of Sulphuric Acid 30 and 200 Against Staphylococcus and Streptococcus Bacteriae

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**Abstract:** *Streptococcus and Staphylococcus are Gram-positive cocci, capable of causing a range of infections, from mild skin infections to more severe diseases. They can also cause a wide range of infections, from localized skin issues like impetigo to more systemic infections such as pneumonia or sepsis. Streptococcus species are well-known for causing strep throat, rheumatic fever, and scarlet fever, while Staphylococcus aureus is notorious for causing skin infections, food poisoning, and more severe conditions like toxic shock syndrome. The preventive efficacy of Sulphuric acid in lesser dilutions is acknowledged by William Boericke in his "Pocket Manual of Homoeopathic Materia Medica and Repertory". This study is aimed to evaluate the antibacterial activities of lower dilutions of homoeopathic medicines Sulphuric acid against Streptococci and staphylococci bacteriae. Thus, it is used in 30C&200C potencies to screen the bacteria by Agar Well-Diffusion assay. Result: Sulphuric acid 30<sup>th</sup> and 200<sup>th</sup> potency doesn't showed any inhibitory activity against Streptococcus by Agar well-diffusion assay but shows zone of inhibition against Staphylococcus bacteria. Conclusion: This experiment clearly showed that the lower dilutions of Homoeopathic medicine Sulphuric acid has antibacterial property against Staphylococcus bacteria.*

**Keywords:** Sulphuric acid, Streptococcus, Staphylococcus, Zone of inhibition

## 1. Introduction

Streptococcus and Staphylococcus are two important genera of gram-positive bacteria, both of which can cause a variety of infections in humans.

Streptococcus are typically spherical and can form chains. Streptococci are gram-positive bacteria that are catalase-negative and coagulase-negative, known for their ability to cause spreading lesions, particularly in infections like cellulitis. They are classified into groups A, B, C, D, and G using the Lancefield system, which is based on specific carbohydrate antigens in their cell walls. Group A (*Streptococcus pyogenes*) is associated with strep throat and skin infections, while Group B (*Streptococcus agalactiae*) is linked to neonatal infections. Additionally, streptococci reproduce through binary fission in three planes, resulting in their characteristic chain or pair formations. They are categorized into groups based on their hemolytic properties—alpha (partial hemolysis), beta (complete hemolysis), and gamma (no hemolysis). Virulent strains on fresh isolation produce a matt colony, while avirulent strains forms glossy colonies. M protein is responsible for the virulence. Typical streptococcal infections are Erysipelas and Impetigo. Common species include *Streptococcus pyogenes*, responsible for strep throat and skin infections, and *Streptococcus pneumoniae*, a leading cause of pneumonia and meningitis. While some species are part of the normal flora, they can become pathogenic, particularly when the immune system is compromised<sup>[1]</sup>

Staphylococcus is a genus of gram-positive, catalase and coagulase positive, spherical bacteria that often forms clusters

resembling grapes. The most notable species is *Staphylococcus aureus*, known for causing a range of infections, from skin issues to severe conditions like pneumonia and septicemia, with (Methicillin-resistant *Staphylococcus aureus* (MRSA) being a resistant strain of concern. Other species, like *Staphylococcus epidermidis*, typically reside on skin but can cause infections in immunocompromised individuals, while *Staphylococcus saprophyticus* is linked to urinary tract infections. These bacteria produce various toxins and enzymes that enhance their virulence and are transmitted primarily through direct contact. The toxins released by the bacteria are enterotoxin responsible for Staphylococcal food poisoning, Exfoliative toxin causing Toxic Shock Syndrome<sup>[2]</sup>. Diagnosis involves culture testing, and treatment often requires antibiotics, though resistance is a significant challenge.

## 2. Literature Survey

- 1) A study conducted to investigate the antibacterial activity of *Hypericum perforatum*, *Arnica Montana*, *Echinacea angustifolia* and *Calendula officinalis* against two strains of bacteria namely *Streptococcus mutans*, *Enterococcus faecalis*. All the homoeopathic medicines used in the form of tinctures exhibited good antibacterial activity against both strains of bacteria, except for *Calendula officinalis* for disc diffusion against *S.mutans* but with *Hypericum perforatum* the highest activity was observed.<sup>[4]</sup>
- 2) Another study was conducted to analyse the efficacy of Homoeopathic and Allopathic medicines on *Staphylococcus aureus* isolated from clinical specimen. The findings of the present study indicate that

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the homoeopathic medicines (BELLADONNA 200c, ARNICA 200c, NUX VOMICA 200c, SULPHUR 200c and BERBERIS VULGARIS 200c) inhibit the growth of bacterium from clinical specimen similar to the allopathic medicines. The study concluded that the Homoeopathic medicines are good substitute of antibiotics for notorious bacterial pathogens like *Staphylococcus aureus* and such medicines neither weaken the immunity system nor produce antibiotic resistant microorganisms as compared with the Allopathic medicines.<sup>[5]</sup>

- 3) In a study it suggests that ultra-diluted homoeopathic preparations at different volumes, particularly lower volumes, exhibited significant antimicrobial activity against *S. aureus* and *E. coli* compared to the negative controls. Therefore, it can be concluded that homoeopathic medicines may be useful in treating disorders caused by antimicrobial resistant bacteria such as *S. aureus* and *E. coli* at lower volumes.<sup>[6]</sup>
- 4) In another study it is confirmed that the Homoeopathic Medicines such as Arsenic album, Antimonium crudum, Hepar sulphur, Silicea has antibacterial activity as seen in Agar well diffusion assay, Minimum Inhibitory Concentration and bactericidal study. Homoeopathic Medicines can be used as alternative medicine for Amikacin and other modern medicines as Homoeopathic Medicines are cheaply available, cost effective and also does not have any side effects. According to results of this study it is seen that Homoeopathic Medicines has anti-bacterial activity against *Staphylococcus epidermidis*.<sup>[7]</sup>

### 3. Materials and Methods

#### Collection of Sample

Drug for the study will be procured from Homoeopathic pharmaceuticals. In this study, Homoeopathic medicine Sulphuric acid 30C and 200C was purchased from Willmar Schwabe which is an approved and standard homoeopathic medicine manufacturing unit.

#### Study setting:

Sarada Krishna Homoeopathic Medical College Research Lab, Kulasekharam

#### Type of study

Experimental in vitro study.

#### Bacterial strains

Standard strains of *Streptococcus* and *Staphylococcus* were used for this study. The culture was grown in Nutrient agar media

#### Antibacterial screening

In this study, Muller Hinton agar plates was used for antibacterial screening.

#### Preparation of disc

Plain sterile disc was purchased from Hi media and soaked with each concentration of extracts and placed at room temperature to get air dry for 6 (six) hrs. Then, the disc paper was labelled and used for antibacterial study.

#### Muller Hinton Agar (MHA):

Nowadays, MHA is more commonly used for the routine susceptibility testing of non-fastidious microorganism by the Kirby-Bauer disk diffusion technique. The agar was prepared by suspending 38gm of the medium in one litre of distilled water. It was then heated with frequent agitation and boiled for one minute to completely dissolve the medium. Followed by Autoclaving at 121°C for 15 minutes and Cooled in room temperature. The cooled solution was then poured into sterile petri dishes on a level, horizontal surface to give uniform depth. And the final pH was then checked. The plates were stored at 2-8 degree Celsius. At the end of incubation, inhibition zones were examined around the disc.

The size of the zone of inhibition (including disc) was measured in millimeter (mm). The absence of zone inhibition was interpreted as absence of activity. The activities were expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm.

#### Methodology

##### Antibacterial activity (kirby-bauer method)

The Antibacterial Activity Was Performed following Kirby-Bauer method. Two Muller Hinton agar plate was prepared and solidified. After Solidification of the Plates the *Streptococcus* and *Staphylococcus* strains was uniformly swabbed Over the Smelted Solidified muller Hinton Plate separately. The plate were undisturbed for 5 minutes, after 5 minutes the plates were taken and the sterile disc 6mm were placed on the appropriate position using sterile forceps. The antibiotic disc (streptomycin 500mg) where also placed ethanol was used as negative control. After few minutes the different potencies (200&30) of homoeopathic medicines was loaded in the sterile disc under sterile condition. 10 µl of the medicine was loaded in each sterile disc. the plate were undisturbed, later the plates were incubated 37°C for 24 hours. After 24hrs. The result will observed by measuring the zone of incubation in millimetres.

### 4. Observation



## 5. Result

The study was done in vitro antibacterial study against staphylococcus and streptococcus with Sulphuric acid 30 and 200 potency. Ethanol was used as negative control and streptomycin 500 mg used as positive control. The Antibacterial Activity Was Performed following Kirby-Bauer method. The Muller hinton agar plate was prepared and solidified. After solidification of the plates the Staphylococcus and Streptococcus bacteriae inoculum was uniformly swabbed over the smolted solidified muller hinton plate. The plate were undisturbed for 5 minutes, after 5 minutes the plates were taken and the sterile disc 6mm were placed on the appropriate position using sterile forceps. Intervension and controls are loaded over the disc denoted as ;

NC: Ethanol (negative control)

PC: Streptomycin 500 mg (positive control)

Bacteria	Zone of Inhibition in mm (Sulphuric Acid 30)	Zone of Inhibition in mm (Sulphuric Acid 200)
STAPHYLOCOCCUS	2.2 mm	2 mm
STREPTOCOCCUS	0	0

The plates were incubated 37°C for 24 hours. After 24hrs the result will observed by measuring the zone of incubation in millimeters. Here I got 2.2 mm and 2mm as zone of inhibition in Sulphuric acid 30 and 200 potency respectively. According to this we can conclude that homoeopathic medicine Sulphuric acid in 30<sup>th</sup> and 200<sup>th</sup> potency is very effective for Staphylococcus infections. I suggest for tinctures and lowest potencies (3C, 6C, 12C etc) which may be effective against Streptococcus bacteria.

## 6. Conclusion

This study conclusively shows that the homoeopathic medicine, Sulphuric acid 30<sup>th</sup> and 200<sup>th</sup> potency can inhibit the growth of Staphylococcus bacteria. Further research on this topic with tinctures and lower potencies should be done to prevent the growth of Streptococcus also as both the Streptococcus and Staphylococcus bacteria are the most common bacterial infections that affects all the systems in human body.

## 7. Future Scope

I suggest for tinctures and lowest potencies (3C, 6C, 12) which may be effective against Streptococcus bacteria.

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