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In-Vitro Study on the Antimicrobial Activity of Various Potencies of Kreosotum Against Staphylococcus Aureus

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ABSTRACT

Staphylococcus aureus is responsible for wide range of infectious diseases with highest morbidity rate. An Antibiotic-resistant Staphylococcus aureus is more in prevalence, it transmits by direct and indirect contact through the discharging wounds or by respiratory and urinary tract. The in-vitro study on the antimicrobial activity of various potencies of Kreosotum against Staphylococcus aureus was carried out in order to evaluate the effectiveness of Kreosotum against Staphylococcus aureus using Disk Diffusion method or Kirby Bauer method. Streptomycin is used as positive control which showed maximum zone of inhibition 13mm and Ethanol as negative control were noticed with no zone of inhibition. Among the various potencies, the Kreosotum 30C showed zone of inhibition of 4mm, whereas Kreosotum 200C showed zone of inhibition of 4mm and Kreosotum tincture showed highest zone of inhibition comparing to other potencies and it was about 9mm. From the study it was conclude that the homoeopathic medicine Kreosotum tincture was effective than other two potencies of Kreosotum.

Keywords: Homoeopathy; Kirby- Bauer disk diffusion; Staphylococcus aureus; Kreosotum

Introduction

Staphylococcus aureus is a Gram-positive spherically shaped bacterium, a member of the Bacillota, frequently found in the upper respiratory tract and on the skin. Staphylococcus aureus can also become an opportunistic pathogen, by causing skin infections including abscesses, respiratory infections such as sinusitis and also food poisoning. Pathogenic strains often promote infections by producing potent protein toxins and the expression of a cell surface protein that binds and inactivates antibodies. It is one of

the leading pathogens for deaths associated with antimicrobial resistance and the emergence of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA)¹.

Drugs Used for the Study

Streptomycin is an antibiotic medicine. It is an aminoglycoside. used to treat a number of bacterial infections including tuberculosis, Mycobacterium avium complex, endocarditis, brucellosis, plague and rat bite fever. It is by injection into a vein or muscle. It works by blocking the ability

of 30S ribosomal subunits to make proteins, which results in bacterial death².

Drug monograph

Source: It is a product of destructive distillation of wood tar

Synonyms: Kreosote, Wood tar

Formula: C₈H₁₀O₂

Description: It is a product of distillation of wood tar and is a mixture of phenols. It is a colourless or faint yellow, strongly refracting liquid.

Active Principles: It is a mixture of phenols. chiefly guaiacol, cresol, methyl cresol and phenol.

Seat of Action: Mucous membrane, gums, teeth, digestive tract, female organs, blood, lymphatic system, skin etc³.

Staphylococcus Aureus

Staphylococcus aureus is Gram-positive bacteria (stain purple by Gram stain) that are cocci shaped and tend to be arranged in clusters that are described as “grape-like.” On media, these organisms can grow in up to 10% salt and colonies are often golden or yellow (aureus means golden or yellow). These organisms can grow aerobically or anaerobically (facultative) and at temperatures between 18°C and 40°C^{4,5}.

Staphylococcus aureus which can be found as part of the normal skin microbiota, in the nostrils^{6,7} and as a normal inhabitant of the lower reproductive tract of females^{8,9} *Staphylococcus aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses¹⁰, septic arthritis, prosthetic device infections, pulmonary infections, gastroenteritis and urinary tract infections¹¹ to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, sepsis. It is still one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery¹⁰.

Streptomycin is an antibiotic medication used to treat a number of bacterial infections. It is administered by injection into a vein or muscle¹². Streptomycin is an antibiotic that inhibits both Gram-positive and Gram-negative bacteria.

Ethanol (also called ethyl alcohol, grain alcohol, drinking alcohol or simply alcohol) is an organic compound with the chemical formula CH₃CH₂OH. It is a psychoactive recreational drug¹³.

Ethanol is used as an antiseptic. Most commonly in medical wipes and in antibacterial hand sanitizer gels for its bactericidal and anti-fungal effects¹⁴. Ethanol kills microorganisms by dissolving their membrane lipid bilayer and denaturing their proteins and is effective against most bacteria, fungi and viruses. However, it is ineffective against bacterial spores, but that can be alleviated by using hydrogen peroxide¹⁵.

Homeopathy Flint as an internal remedy is practically unknown. Hahnemann introduced it into medicine and it was his method of attenuating insoluble substances that enabled him to discover its powers.

Kreosotum the homeopathic medicine is a mixture of phenols obtained from this distillation. Kreosotum acts on all mucus membranes more especially in digestive organs, producing catarrhal condition, with tendency to disorganization

and destruction of tissue. Great acidity of all secretions. Excoriating, burning and offensive discharges. Hæmorrhage, ulcerations, cancerous affections. Rapid decomposition of fluids and secretions and burning pains¹⁶ and Kreosotum prevents secretion of lymphatic system and produce great acidity of all secretions. Acrid and excoriating uterine secretions and putrid ulceration.

Therapeutic individuality

The menses are usually too frequent and too profuse, succeeded by an acrid-smelling, bloody ichor, with corrosive itching and biting of the parts, “Acrid, bloody ichor from the womb, with corrosive itching of the parts; stitches in the vagina.” Yellow, acrid, putrid, excoriating leucorrhœa.

Leucorrhœa, with great debility. It may be mild or acrid, causing great itching. “Very offensive, excoriating lochia; almost ceasing, then it freshens up again.” [Excoriating the vulva] “Her hæmorrhage seems to pass into a corrosive, ichorous discharge; then to freshen up again and go on.” Corrosive itching within the vulva and itching between the labia and thighs, with soreness and burning after urinating. Extreme soreness of the labia. “Voluptuous itching deep in the vagina.”

Burning and swelling of external and internal labia, awful burning, as of red-hot coal in the pelvis, with discharge of clots of foul-smelling blood.

Chronic keratitis, with excessively hot, smarting lachrymation and moderately profuse, excoriating discharge. Intense itching of the margins of the eyelids; lids much swollen with pustular eruptions over the whole body, with great itching.

Wretched skin, livid complexion, pustular eruption over the whole body.

“Herpes; humid, scaly, pustular; on the eyelids, cheeks, mouth, elbows, fingers, malleoli; or large greasy, pox-shaped pustules all over the body.” The itching in the evening became so violent that she was almost crazy. [17] Gangrenous ulceration.

Hæmorrhage from the bowels, bleeding from the mucous membranes. The mouth becomes raw and wherever there is a mucous membrane there is a rawness and the fluids that ooze continue to eat and cause ulceration. Vomiting, bleeding, diarrhœa and the fluids vomited from the stomach are so acrid that they seem to take the skin off from the mouth, make the lips raw. So, excoriation from acrid fluids¹⁸.

The previous study conducted by TC Divya, Sapna Chandira Muddappa, VP Prabath Singh, Rakesh R Rajan, Arjun B Ravi on antimicrobial efficacy of Homeopathic medicines Acid Carbohc, Kreosotum, Echinacea and Thuja occidentalis against *Enterococcus faecalis* biofilm Formed on tooth using disc diffusion method the result was observed in acid carbohc and *E. angustifolia*¹⁹.

The previous study conducted by Deresse Daka on in vitro study to know the antibacterial effect of garlic (*Allium sativum*) on *staphylococcus aureus* by using agar dilution method using Muller Hinton agar and the results was stated that Garlic (*A. sativum*) has antimicrobial properties against *Staphylococcus aureus*. It has both a bacteristatic and bactericidal activity. When tested in vitro using crude preparation of garlic²⁰.

The previous study conducted by Shifa Shaffique, Haseeb Anwer, Hafiz Muhammad Asif, Muhammad Akram, Anila

Rehman, Saeed Ahmed, Khalil Ahmed on In vitro study of evaluation of antioxidant activity of homeopathic mother tincture of plant origin i.e [Baptisia tinctoria, Berberis aquifolium, Echinacea angustifolia, Hydrangea arborescens, Hydrastis canadensis, Kreosotum and Thuja occidentalis] in comparison with ascorbic acid and total phenolic content by disc diffusion method and the results observed were plant origin showed significant antioxidant potential except Hydrangea arborescens and Kreosotum²¹.

The previous study conducted by Pooja Agarwal, L Nagesh on Evaluation of the antimicrobial activity of various concentrations of Tulsi (Ocimum sanctum) extract against Streptococcus mutans by an in vitro study by disc diffusion method and the results observed was the zone of inhibition was more in ocimum sanctum and Streptococcus mutans was resistant to the action of Tulsi extract²².

The previous study conducted by Tanmay Sarkar, Pashmin Kaur Anand, Arun Bhargav Jadhav, Bipinraj Nirichan Kunchiraman, Chetan Hanamantrao Shinde, Pune Dhankawadi on in-vitro study of anti-bacterial activity of homeopathic medicine Sulphanilamide against Staphylococcus epidermidis by disc diffusion method and the results observed was nearly all the potencies are showed antibacterial activity. The effectiveness of Homoeopathic medicine was seen by observing the zone of inhibition, which Range from 0.6 to 0.8 mm for different potencies²³.

Aims and Objectives

Aim

To study the antimicrobial effects of various potencies of Kreosotum against Staphylococcus aureus.

Objectives

To determine the effect of Kreosotum against Staphylococcus aureus in the culture medium. 2. To compare the effect of various potencies of Kreosotum 30C, 200C, Q against, Staphylococcus aureus over Streptomycin.

Materials and Method

Selection of sample

The drugs for the study are procured from Bahola Homoeopathic Pharmaceutical Company. Pure culture of Staphylococcus aureus is collected from MTCC (ATCC 25923).

The culture medium purchased from high media

Sensitivity discs: disc of 5mm diameter of Whatman Filter Paper.

Study setting

Microbiology Lab, Research Facilitation Centre, Sarada Krishna Homoeopathic Medical College, Kanyakumari.

Type of study

Experimental In-vitro study.

Method of collection of data

Conducting the study / obtaining data by disc diffusion method or Kirby-Bauer testing method on culture medium.

The drugs for conducting the study are Kreosotum 30C, 200C, Q and Streptomycin as positive control.

These are divided into five groups:

Group I - Streptomycin antibiotic as positive control group

Group II - Ethanol as negative control group

Group III - Kreosotum 30C Group IV - Kreosotum 200C

Group V - Kreosotum Q

Methodology

Kirby Bauer Method

Antibacterial activity of sample was determined by Disk Diffusion method on Muller Hinton Agar (MHA) medium. The Muller Hinton Agar medium was weighed as 3.8 gm and dissolved in 100ml of distilled water and add 1 gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petri-plates and were allowed to solidify for 1 hour. After the medium was solidified, the inoculums were spread on the plates with sterile swab moistened with the bacterial suspension. Streptomycin tablet is placed on MHA plate as positive control. Four plain discs medicated with various potencies of Kreosotum (30C, 200C and tincture) and Ethanol as negative control were placed on MHA medium. This plate was incubated for 24 hours at 37°C. Then the microbial growth was determined by measuring the diameter of zone of inhibition around the disc (Figures 1-3).



Figure 1: MH plates.



Figure 2: Preparation of bacterial lawn.

Observation and Results

When comparing the antibacterial activity of Homoeopathic medicine Kreosotum with ethanol and antibiotic streptomycin 10 mcg. The antibiotic showed the maximum zone of inhibition of 13 mm and ethanol have no zone of inhibition that is zero (Figures 4,5).

Among three potencies of Kreosotum 30C, 200C and tincture. Kreosotum tincture showed the antibacterial activity with zone of inhibition of 9mm. The Kreosotum 200C and 30C showed zone of inhibition of 4mm (**Table 1**).

Hence, Homoeopathic medicine Kreosotum tincture showed anti-bacterial activity against *Staphylococcus aureus*.

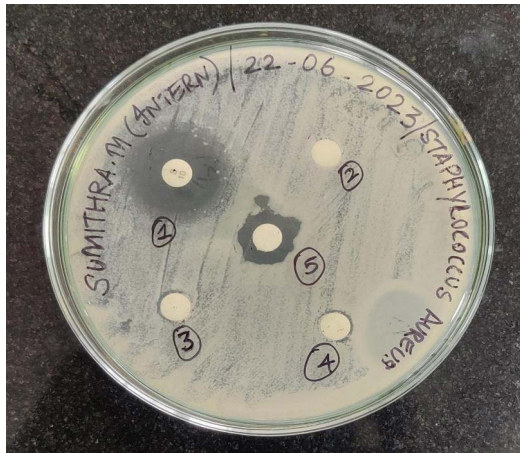


Figure 4: Anti-bacterial activity of Kreosotum.

Table 1: Antibacterial activity of Kreosotum against *Staphylococcus aureus* By tabulating the zone of inhibition.

DISC	DIAMETER OF ZONE OF INHIBITION
Streptomycin [Positive control]	13 mm
Ethanol Negative control]	0 mm
Kreosotum 30	4 mm
Kreosotum 200	4 mm
Kreosotum Q	9 mm

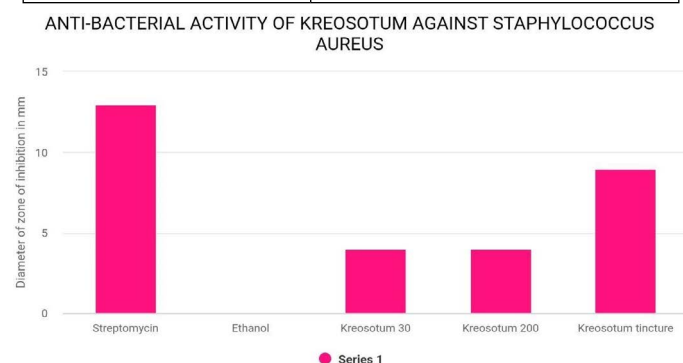


Figure 5: Diagrammatic representation of result of antibacterial activity of Kreosotum 30C, 200C & Kreosotum Q against *Staphylococcus aureus* by Disk diffusion method

Discussion

Staphylococcus aureus is a gram-positive bacterium whose treatment remains challenging due to the rapid emergence of antibacterial resistance in the past two decades showing rapid shift in epidemiology of its infections. In this study while comparing the antibacterial activity of Homoeopathic medicine Kreosotum with antibiotic streptomycin 10mcg, antibiotic showed the maximum zone of inhibition of 13mm and ethanol showed no zone of inhibition whereas among three potencies of Kreosotum 30C, 200C and tincture. The Kreosotum tincture showed antibacterial activity with zone of inhibition of 9mm and 30C, 200C showed the zone of inhibition of 4mm.

Homoeopathic system of medicine is based on certain laws and principles. Though this system is used by millions of people around the world, the scientific community still consider this as a controversial topic. This is partly due to the inconsistency of its laws with fundamental scientific concepts and partly due to the absence of any detectable active ingredient in highly diluted preparations. From this research it is evident that homeopathic medicines are not placebos. However, identification of active compounds presents in the homoeopathic formulations and how homoeopathic medicines work to inhibit the growth of *staphylococcus aureus* in vitro has not been evaluated in this study which should be subjected to further investigations.

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