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An *in-vitro* study on antibacterial activity of *Croton tiglium* and their potencies against *Staphylococcus aureus*

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Abstract
A common component of the body's microbiota, *Staphylococcus aureus* is a spherically shaped, Gram-positive bacteria that belongs to the Bacillota and is commonly found on the skin and in the upper airways. It is an anaerobe with facultative characteristics that can grow without oxygen and frequently tests positive for nitrate reduction and catalase. *S. aureus* typically functions as a symbiont of the human microbiota, but it can potentially turn into an opportunistic pathogen. It is frequently responsible for food poisoning, respiratory infections like sinusitis, and skin infections like abscesses. By generating virulence characteristics including strong protein toxins and the production of a cell-surface protein which binds and deactivates antibodies, pathogenic strains frequently encourage infections. Generally speaking, the skin and mucus membranes provide a strong defense against infection. However, *S. aureus* may enter the tissues underneath or the circulation if those barriers are compromised (for example, by trauma-induced skin injury or viral infection-induced mucosal damage). Abscesses, cellulitis, food poisoning, osteomyelitis, and septicemia are just a few of the many illnesses that *S. aureus* is a major cause. Approximately one-third of healthy people have these germs on their skin, in their pharynx, and in their noses. The purpose of this study is to assess *Croton tiglium* Q's antibacterial properties at 30 and 200 degrees Celsius against *Staphylococcus aureus* bacterial strains. The positive control was the antibiotic streptomycin, while the negative control was ethanol. The zone of inhibition demonstrated good antibacterial properties against the Staphylococcal aureus bacterial strains when tested for the various potencies of the bacteria.

Keywords: Antibacterial property, *Croton tiglium*, *Staphylococcus aureus*, zone of inhibition

Introduction
Staphylococci are the most ubiquitous organisms affecting man. Staphylococci are gram-positive cocci 1 um in diameter, capable of aerobic and anaerobic metabolism. They produce lesions by direct invasion. Many strains are toxigenic and several syndromes result from toxic effects. Incubation period for local lesions is 2-3 days. *Staphylococcus aureus* is coagulase positive. Healthy people carry staphylococci in the nose and to a lesser extent in the perineum. The organism is spread by direct contact, through fomites, dust or by airborne droplets. Large number of organisms are shed from superficial lesions and from the respiratory tract [1]. The natural human flora includes *S. aureus*, which can colonize 25-50% of healthy individuals either permanently or temporarily. Insulin-dependent diabetics, HIV-positive patients, hemodialysis patients, and people with skin injury had greater colonization rates [3].

All things considered, *S. aureus* is a major contributor to nosocomial infections. It is the second most frequent cause for primary bacteremia after CoNS and the most frequent cause of operational wound infections. Nosocomial isolates are becoming more and more drug-resistant. *S. aureus* continues to be a significant cause of respiratory infections, infections of the skin and soft tissues, and infective endocarditis in injectable drug users. Another factor contributing to community-acquired staphylococcal infections is the growing use of home infusion therapy [3]. Staph. aureus growth in blood culture shouldn't be written off as a "contaminant" until all potential underlying source have been thoroughly ruled out and a subsequent blood culture comes back negative [2].

Pus-filled blisters, which frequently start within hair follicles and propagate to nearby tissues, are the hallmark of these illnesses. Folliculitis refers to a superficial infection of the

hair follicle, characterized by erythema and induration surrounding a core region of purulence (pus). Furuncles, also known as boils, are larger, more painful lesions that typically appear in moist, hairy areas of the body. They start from hair follicles and grow into a genuine abscess with a core purulence. The coalescence of additional lesions which extend to a deeper layer within the subcutaneous tissue causes carbuncles, which are more severe and painful and are typically found in the lower neck [3].

The purpose of this investigation is to ascertain *Croton tiglium*'s antibacterial efficacy against *Staphylococcus aureus* germs. We can determine whether or not *Croton tiglium* is an effective treatment for *Staphylococcus aureus* by measuring the inhibitory zone. Because homoeopathy functions on a dynamic basis, we can demonstrate that it can treat *Staphylococcus aureus* instances, even if they are treatment resistant.

Materials and Methods

1. Collection of the samples

The study's medication will be purchased from homoeopathic pharmacies. Sharda Boiron Laboratories (SBL) Ltd., an authorized and standard homoeopathic medicine production facility, provided the homoeopathic medicine *Croton tiglium* Q, 30C, and 200C used in this investigation.

2. Setting for study

Research Lab of Sarada Krishna Homoeopathic Medical College Kulasekharam

3. Type of the study

An *in vitro* experimental Study.

4. Bacterial strains

a. For this investigation, standard strains of Staphylococcal aureus were employed. Muller Hinton agar medium was used to cultivate the culture.

b. Bacterial strain resuscitation

A few bacterial colonies from a 24-hour-old pure culture plate were removed and floated in nutrient broth to create the bacterial inoculum. Next, a concentration of 1×108 CFU/ml was set for the solution. The turbidity of the solution being tested at this concentration was equal to the McFarland standard of 0.5 (OD) at 620 nm. The bacterial inoculum was kept at 4°C. This culture used as an inoculum for additional research.

5. Antibacterial screening

Muller Hinton's agar plates were utilized in this investigation to test for bacteria.

6. Disc preparation

After purchasing a plain sterile disc from Hi Media, it was soaked in each extract concentration and allowed to air dry for six hours at room temperature. The disc paper was then marked and put to use in an antibacterial investigation.

7. Method of collection of data

The drugs used for conducting the study are *Croton tiglium* Q, 30C and 200C.One positive control is kept, which is Streptomycin 10 mg. One negative control is also kept, which is ethanol. Thus, five groups are formed which are shown in the following table.

Table 1: Samples and groups

Groups	Samples
I	*Croton tiglium* Q
II	*Croton tiglium* 30C
III	*Croton tiglium* 200C
IV	Positive control - Streptomycin 10mg
V	Negative control - Ethanol

8. Muller hinton's agar (MHA)

These days, MHA is more frequently employed in the Kirby-Bauer disk diffusion method for regular susceptibility testing of non-fastidious microorganisms. 38 g of medium were suspended in one liters of distilled water to create the agar. The medium was then thoroughly dissolved by heating it with continuous stirring and boiling it for a minute. After autoclaving for 15 minutes at 121°C, it was cooled to room temperature. In order to achieve consistent depth, the cooled solutions was then transferred into a sterilized petri dish on a flat, horizontal surface. After that, its final pH was measured. The plate was kept between 2 and 8 degrees Celsius. Inhibition zones surrounding the disc were investigated after the incubation period. Millimetres (mm) were used to measure the zone of inhibition's size, including the disc. It was assumed that there was no activity because there was no zone inhibition. The activities were classified as sensitive if the zone of inhibition was greater than 11 mm, moderate if it was between 8 and 10 mm, and resistant if it was less than 7 mm [5].

9. Methodology

ANTIBACTERIAL ACTIVITY (Kirby-Bauer method)

The Kirby-Bauer method was used to carry out the antibacterial activity. After preparation, the Muller Hinton's Agar plate solidified. The *Staphylococcus aureus* inoculum was evenly swabbed across the solidified Muller Hinton's plate once the plates had set. After the plate had remained undisturbed for five minutes, sterile forceps were used to put the 6 mm sterile disc in the proper location. The positive control was 10 mg of streptomycin, while the negative control was ethanol. The various potencies (Q, 30, and 200) of the homoeopathic remedy *Croton tiglium* were poured into the sterile discs under sterile conditions after a few minutes. Each sterilized disk contained 10 µl of the medication. After being left undisturbed, the plate was incubated for 24 hours at 37˚C. The zone of incubation will be measured in millimeters to determine the outcome after 24 hours [5].

Results

Croton tiglium was used in an *in vitro* antibacterial investigation against *Staphylococcus aureus* at several potencies, including Q, 30C, and 200C. The positive control was 10 mg of streptomycin, while the negative control was ethanol. The Kirby-Bauer method was used to carry out the antibacterial activity. The discs were identified in the plates according to the table that follows.

Table 2: Samples and their codes used

CODE	SAMPLES
CT Q	*Croton tiglium* Q
CT 30	*Croton tiglium* 30C
CT 200	*Croton tiglium* 200C
PC	Positive control - Streptomycin 10mg
NC	Negative control - Ethanol

The plate was incubated for 24 hours at 37˚C. The zone of the incubation was measured in millimeters to assess the results after a day. In this case, I received 8 mm for positive control, 5 mm for Q, 3 mm for 30C, and 6 mm for 200C for *Croton tiglium*. Thus, by my experimental study, it is shown that *Croton tiglium* shows mild to moderate inhibitory action against the *Staphylococcus aureus* bacteria. Hence, it will be effective in treating patients affected with *Staphylococcus aureus* and will help in curing them homoeopathically.

Picture 1: After 24 Hours - Zone of inhibition

Table 3: Zone of inhibition values

Samples	Zone of inhibition
Positive control - Streptomycin 10 mg	8 mm
Negative control - Ethanol	0 mm
Croton tiglium Q	3 mm
Croton tiglium 30C	1 mm
Croton tiglium 200C	6 mm

Discussion

The emergence of antibiotic-resistant strains of *Staphylococcus aureus* has led to the development of alternative therapeutic approaches, and homoeopathy presents promising results in this area. *Croton tiglium*, commonly known as purging croton, has gained attention for its diverse pharmacological properties, such as anti-bacterial, anti-inflammatory, anti-tumor, anti-HIV and Analgesic activity [10].

Considering *S. aureus*'s high virulence, wide range of infections, and capacity to cause substantial mortality and morbidity, the medical community's greatest issue is coming up with effective new ways to combat this danger because traditional treatments are frighteningly ineffective [4]. Conventional medicine primarily relies on antibiotics to combat bacterial infections and the escalating rates of resistance have necessitated exploration beyond these antibiotics. Homeopathy, with its principle of "like cures like" offers a unique approach to cure the patients. Homoeopaths treat not just the disease but the patient as a whole [9]. The inclusion of *Croton tiglium* as a homeopathic remedy in the treatment of Staphylococcal aureus infections aligns with this philosophy, capitalizing on the antimicrobial properties of *Croton tiglium*.

The negative control ethanol that I have used shows no inhibitory action, but our medicine's inhibitory value was high. Hence, it is proved that our medicine has separate action apart from the action of its solvent used for dilution. My experimental study suggests the potential of *Croton tiglium* in inhibiting Staphylococcal aureus bacterial growth and provides a foundation for further research in this domain.

Conclusion

From this study, it is shown that *Croton tiglium* Q, 30C and 200C shows moderate antibacterial activity against the *Staphylococcus aureus* bacteria. They showed 3 mm, 1 mm and 6 mm of inhibition zone respectively. There is no chance of developing drug resistance for homoeopathic remedies, as it acts dynamically. Hence, it is crucial to acknowledge the scope of *Croton tiglium* in homoeopathic treatment of Staphylococcal infections. This requires rigorous clinical trials and systematic studies to establish its efficacy. The exploration of *Croton tiglium* in homeopathy offers hope against bacterial infections, underscoring the importance of synergistic approaches in contemporary healthcare.

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Conflict of Interest

Not available

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Not available

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