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A STUDY TO EVALUATE THE ANTIBACTERIAL ACTIVITY OF HOMOEOPATHIC MEDICINE HYDROCOTYLE ASIATICA MOTHER TINCTURE 30th POTENCY AND 200th POTENCY AGAINST STAPHYLOCOCCUS AUREUS – IN VITRO

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Hydrocotyle asciatica, staphylococcus aureus, Zone of inhibition Kirby-bauer diskdiffusion.

ABSTRACT

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 meansgolden or yellow. This study is aimed to evaluate the Antibacterial activity of Homoeopathic medicine Hydrocotyle asciatica mother tincture, 30th and 200th potency against staphylococcus aureus.

Methodology

a. Bacterial Strains:

Standard strains of staphylococcus aureus were used for this study. The culture wasPurchased from Sarada Krishna Homoeopathic Medical College and Hospital, Kulasekharam.

b. Resuscitation of Bacterial Strains:

For the preparation of bacterial inoculum, few colonies of bacteria were taken from 24-hr- old pure culture plate and suspended into nutrient broth. Then, solution was set for concentration of 1× 108 CFU/ml. At this concentration, turbidity of the solution was equivalent to 0.5 (OD) at 620 nm McFarland standard. Bacterial inoculum was stored at 4◦C. This culture was used as an inoculum for further studies.

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Result: `Hydrocotyle asciatica tincture – 2 mm, 30C - 4mm, 200C - 8 mm, Hydrocotyle asciatica in 200C showed maximum Growth inhibitory zone (GIZ) against staphylococcus aureus after comparison with Vehicle control (Ethanol 90%) and Positive control (Streptomycin10mg)

Conclusion: This experiment clearly showed that the homoeopathic medicine `Hydrocotyle asciatica has antibacterial property against staphylococcus aureus.

INTRODUCTION

Staphylococcus aureus is a Gram-positive spherically shaped bacterium, a member of the Bacillota, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen.[1] Although *S.* aureus usually acts as a commensal of the human microbiota, it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies.[1] In 1880, Alexander Ogston, a Scottish surgeon, discovered that Staphylococcus can cause wound infections after noticing groups of bacteria in pus from a surgical abscess during a procedure he was performing. He named it Staphylococcus after its clustered appearance evident under a microscope. Then, in 1884, German scientist Friedrich Julius Rosenbach identified Staphylococcus aureus, discriminating and separating it from Staphylococcus albus, a related bacterium. In humans, S. aureus can be present in the upper respiratory tract, gut mucosa, and skin as a member of the normal microbiota. S. aureus produces various enzymes such as coagulase (bound and free coagulases) which facilitates the conversion of fibrinogen to fibrin to cause clots which is important in skin infections. Hyaluronidase (also known as spreading factor) breaks down hyaluronic acid and helps in spreading it.

Deoxyribonuclease, which breaks down the DNA, protects S. aureus from neutrophil extracellular trap-mediated killing. S. aureus also produces lipase to digest lipids, staphylo-kinase to dissolve fibrin and aid in spread, and beta- lactamase for drug resistance.

The carriage of S. aureus is an important source of hospital-acquired infection (also called nosocomial) and community-acquired MRSA. Although S. aureus can be present on the skin of the host, a large proportion of its carriage is through the anterior nares of the nasal

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passages and can further be present in the ears. The ability of the nasal passages to harbour S. aureus results from a combination of a weakened or defective host immunity and the bacterium's ability to evade host innate immunity. Nasal carriage is also implicated in the occurrence of staph infections. *S.* aureus acquired from an external source could be the cause of an infection when inoculated into an open wound. More commonly, the human host is infected by bacteria that colonize her or his skin or mucosal surface. The mucosal surfaces that harbor *S.* aureus include the nose, throat, vaginal wall, and GI tract. Nasal carriage is probably most important because nose-picking could effectively disseminate the bacterium to other body surfaces and other hosts. Remarkably, 20% of individuals are persistently colonized in the nose and 30% are transiently colonized. *S.* aureus has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most staph infections are not serious, *S.* aureus can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infections.[2] Hydrocotyle: Botanical name: Centella asiatica ;Common name: Indian pennywort; Family: Umbelliferae ; Part used: Whole plant

Curative in disorders that exhibit interstitial inflammation and cellular proliferation in any part. Hypertrophy and induration of connective tissue. Has considerable reputation in leprosy and lupus, when there is no ulceration. The skin symptoms are very important. Of great use in ulceration of womb. Very copious perspiration. Pains of cervical cancer.(3) Female: Pruritus of vagina. Inflammation of neck of bladder. Heat within vagina. Granular ulceration of womb. Profuse leukorrhea. Dull pain in ovarian region. Cervical redness. Skin: Dry eruptions. Great thickening of epidermoid layer and exfoliation of scales. Psoriasis gyrates, on trunk and extremities, palms and soles. Pustules on chest. Circular spots, with scaly edges. Intolerable itching, especially of soles. Profuse sweat. Syphilitic affections. Acne. Leprosy. Elephantiasis. Lupus non-exedens.

REVIEW OF LITERATURE:

IN-VITRO ANTIBACTERIAL POTENTIAL OF HYDROCOTYLE:

In this study, in vitro antibacterial assay of the methanol extract of Hydrocotyle was carried out by a well diffusion method. It was observed that the methanol and water extracts had wide antibacterial activity against both Gram positive and Gram-negative bacterial strains under study. This spectrum of antibacterial activity showed that the methanol and water extracts contained some active constituents that could be used against food spoilage,

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gastroenteritis and topical bacterial pathogens.[4]

ANTIMICROBIAL ACTIVITY OF CENTELLA ASIATICA AND GIGANTOCHLOA APUS:

In this study the ethanol extracts of C. asiatica, and G. apus shoot were obtained by using speed extractor, The ethanol extract's antimycobacterial activity was determined using Lowenstein Jensen medium and antibacterial activity was determined using Kirby-Bauer methods on Mueller Hinton agar (MHA). The phytochemical analysis showed that C. asiatica extract contains flavonoids, alkaloids, saponins, and tannins and C. asiatica showed antimicrobial activity against all pathogenic bacteria tested except B. subtilis. (5) This study is intended to know more about the antibacterial effect of Hydrocotyle.

MATERIALS AND METHODOLOGY

a. Bacterial Strains

Standard strains of staphylococcus aureus were used for this study. The culture was Purchased from Sarada Krishna Homoeopathic Medical College and Hospital, Kulasekharam.

b. Resuscitation of Bacterial Strains

For the preparation of bacterial inoculum, few colonies of bacteria were taken from 24-hr- old pure culture plate and suspended into nutrient broth. Then, solution was set for concentration of 1× 108 CFU/ml. At this concentration, turbidity of the solution was equivalent to 0.5 (OD) at 620 nm McFarland standard. Bacterial inoculum was stored at 4◦C. This culture was used as an inoculum for further studies.

Homoeopathic Medicine

In this study, Hydrocotyle asiatica was purchased from wilmar schwabe which is an approved and standard homoeopathic medicine manufacturing unit.

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 hrs. Then, the disc paper was labeled and used for antibacterial study.

Mueller 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

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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 millimetre (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.

OBSERVATION

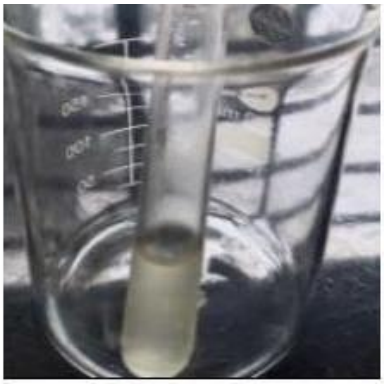

FIGURE 1: Staphylococcus aureus culture

FIGURE 2: Staphylococcus aureus inoculum was uniformly swabbed over the solidified MH plate

FIGURE 3: Using sterile forceps 6mm disc were placed on MH plate

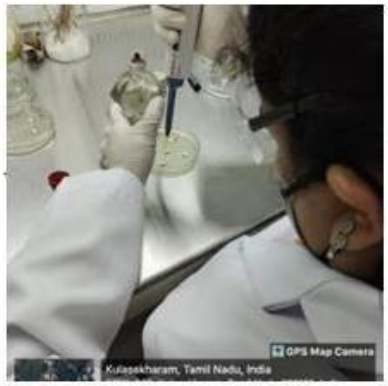

FIGURE 4: Adding homoeopathic medicine

FIGURE 5: After 24 hours of medical suspension

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TABLE 1: Zone of Inhibition of staphylococcus aureus and Control by Kirby-Bauer Method

DISC	DIAMETER OF INHIBITION ZONE (MM)
PC	7 mm
NC	0 mm
30C	4 mm
200C	8mm
TIN	2mm

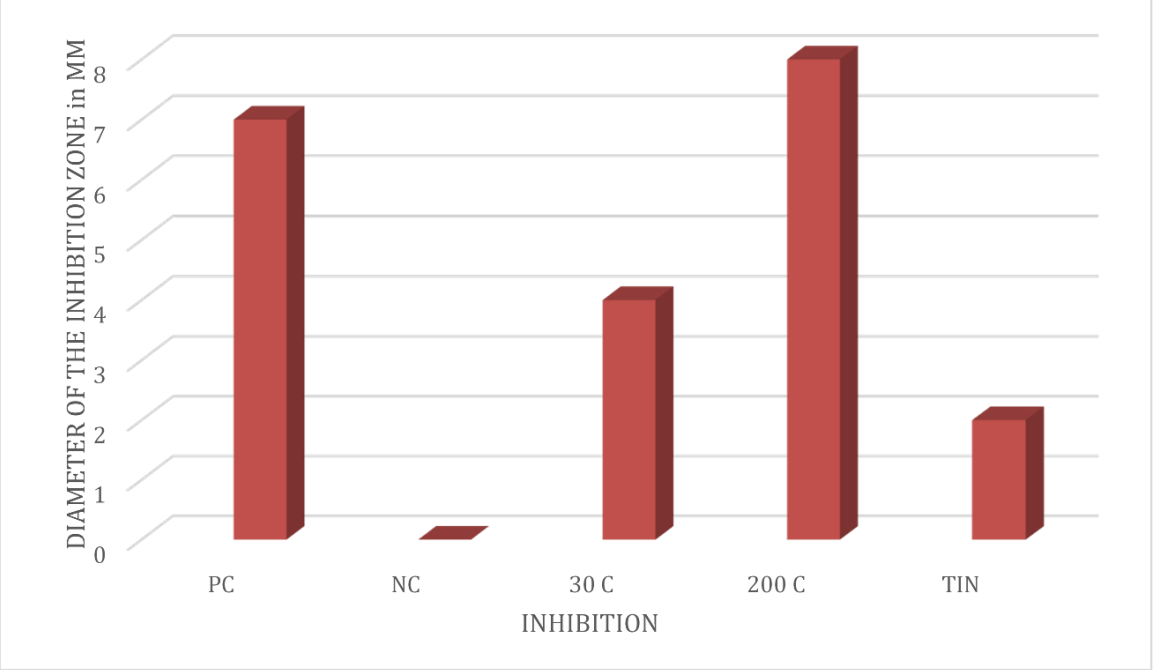

FIGURE 6 Diameter of the Inhibition Zone in MM

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RESULT

The study was done in vitro antibacterial study by using Hydrocotyle asiatica in two different potencies namely 30C, 200C and Mother tincture against staphylococcus aureus. Ethanol was used as negative control and Streptomycin 10 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 aureus inoculum was uniformly swabbed over the smolted solidified muller hinton plate. The plate were undisturbed for 5 min, after 5 minutes the plates were taken and the sterile disc 6mm were placed on the appropriate position using sterile forceps. Intervention and controls are loaded over the disc denoted as; HA : Hydrocotyle asiatica

NC: Ethanol (negative control)

PC: Streptomycin 10 mg (positive control)

The plates were incubated 37 for 24 hours. After 24hrs the result will observed by measuring thezone of inhibition in millimeters.

Here I got Zone of inhibition as:

Hydrocotyle asiatica 30 –	zone of inhibition: 4 mm
Hydrocotyle asiatica 200 –	zone of inhibition: 8 mm
Hydrocotyle asiatica mother tincture –	zone of inhibition: 2 mm
Positive control (Streptomycin 10mg) –	zone of inhibition: 7 mm
Negative control (Ethanol) –	zone of inhibition: 0 mm

According to this we can concluded that homoeopathic medicine in Hydrocotyle asiatica 200th potency is effective against Staphylococcus infection other than mother tincture.30th potency alsohas action comparatively

DISCUSSION

S.aureus is a leading bacterial cause of a wide range of infections, including abscesses, cellulitis, food poisoning ,Osteomyelitis and septicaemia. Around one third of healthy individualscarry this bacterium in their noses, pharynx and on their skin.

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Cellulitis is One of the common diseases among community, which enter through a crack or break in the skin. The incidence of a more serious staphylococcus infection called methicillin- resistant Staphylococcus aureus (MRSA) is increasing. Cellulitis can occur anywhere on the body, but the most common location is the lower leg. Bacteria are most likely to enter broken, dry, flaky or swollen skin, such as through a recent surgical site, cuts, puncture wounds, ulcers, athlete's foot or dermatitis. Most times, taking antibiotics cures cellulitis. However, some people can have serious complications. Without treatment, it can become life-threatening.(6)

Infectious diseases are a major cause of morbidity and mortality worldwide. The use of antibiotics to control these diseases has led to the emergence of antibiotic-resistant pathogens and, therefore, the need for alternative medicines, such as medicinal plants, which are easily available,affordable, and efficacious with minimal side effects

Bacterial resistance to currently used antibiotics necessitates the search for effective therapeutic agents. The use of medicinal plants presents a great potential as a source of antimicrobial compounds against resistant pathogenic microorganisms

It is always difficult to choose a selective antibiotic on the first day of treatment as we don't know about the causative organism and its sensitivity. To find the causative organism and its sensitive antibiotics, clinicians need sputum culture and sensitivity. For culture and sensitivity, it might take minimum of 48-72hr. In that period of time the patient health may detoriate. Hence it is necessary to start with empirical antibiotic by finding the causative organism just with its characteristic symptom.[7]

Homoeopathic medicines provide a good empirical antibiotic activity for many organisms as well as allopathic medication. This study focused on 3 potencies of a remedy hydrocotyle asciatica remedy an empirical homeopathic antibiotic agent for a selected organism – staphylococcus aureus, this study to evaluate the ability of its different potencies (30C, 200C and mother tincture) to inhibit S.aureus in vitro.(8)

The selected Medicine with different potencies is placed in growth agar for sensitivity test and more zone of inhibition is observed on Hydrocotyle200C. In this experiment Homoeopathic medicine have shown better growth inhibition zone (GIZ) than Vehicle control (ethanol 90%). Although alcohol with strength 60% to 90% v/v has known for its antibiotic properties and Positive control of Amoxyclavatum used. Hydrocotyle asiatica has been utilized traditionally for the treatment of infectious disease.

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It is used to treat wounds, mental and neurological disorders, atherosclerosis, microbial infections, and cancer. It is also used in the treatment of inflammations, diarrhoea, asthma, tuberculosis, and various skin lesions and ailments such as leprosy, lupus, psoriasis, and keloid. It is reported to possess ulcer-preventive, antioxidant, and antidepressive effects and improves venous insufficiency.

Hydrocotyle asciatica is Curative in disorders that exhibit interstitial inflammation and cellular proliferation in any part. Hypertrophy and induration of connective tissue. Has considerable reputation in leprosy and lupus, when there is no ulceration. The skin symptoms are very important. Of great use in ulceration of womb. Difficulty in maintaining the upright posture. Very copious perspiration. Pains of cervical cancer.[9]

In the six edition of organon according to Aphorism 27 ; Hahnemann said that the curative power of medicine, therefore, depends on their symptoms, similar to the disease but superior to it in strength (§12-§26),so that each individual case of disease is most surely, radically, rapidly and permanently annihilated and removed only by a medicine capable of producing (in the human system)in the most similar and complete manner the totality of its symptoms, which at the same time are stronger than the disease.[10]

This study also shows that the zone of inhibition of the bacteria, staphylococcus aureus by Homoeopathic medicine was not due to the ethanol, some distinct dynamic pharmacological actions were behind it.

CONCLUSION

This study conclusively shows that the homoeopathic medicine Hydrocotyle asciatica tincture,30, &200, particularly 200 potencies, can inhibit the growth of S. Aureus. If we can limit bacterial infections through homoeopathic medicines, it can be helpful to control childhood infections and can also be used to reduce the use of antibiotics and its complications because the human body exhibits high affinity for staphylococcus aureus infections from the time of birth. Further research on this topic should be done for the benefit of humanity as it is a new finding.

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4. Author links open overlay panelManab Mandal a, a, b, c, d, AbstractObjectiveTo explore the in vitro antimicrobial potential of Hydrocotyle javanica (H. javanica) Thunb. against human pathogenic bacteria and to standardize purification protocol to harvest the active principle in large scale.MethodsIn the present s, et al. In vitro antibacterial potential of hydrocotyle javanica thunb.

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