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Investigating the pharmaceutical aspects of infused oil of *Hibiscus sabdariffa* flower against folliculitis caused by gram positive *Staphylococcus aureus.*

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Abstract: Treatment and prevention of illnesses of the hair and scalp have benefited greatly from the use of natural medications when used in conjunction with traditional medical procedures. Staphylococcus aureus, one of the four most common folliculitis bacteria, was tested for antimicrobial susceptibility in hibiscus oil using the agar well diffusion technique. The minimum inhibitory concentration (MIC) was calculated using the quantity of hibiscus oil required to treat one milliliter of agar solution as the input. Significant antibacterial activity against Staphylococcus aureus is shown by the plant extract. It was shown that the 300 mg/ml Minimum Inhibitory Concentration (MIC) was effective against both strains of Staphylococcus aureus. According to the findings, hibiscus oil effectively inhibits bacterial infections in hair follicles and reduces inflammation.

Keywords: *Hibiscus sabdariffa, flower oil, Staphylococcus aureus,* folliculitis, hair growth, scalp problems

Introduction: Folliculitis Decalvans (FD) is a persistent inflammatory condition with no recognized cause. It is believed that *Staphylococcus Aureus*, which is usually present on lesional skin, plays a causative role, but the significance of its involvement is still debatable. We used culture techniques, genetic identification, and *S-aureus* virulence factors to compare the superficial and subepidermal microbiota in twenty FD patients with lesional skin containing S aureus and in twenty healthy controls prior to and after an anti-staphylococcal treatment. In 80% of instances, S aureus colonised non-lesional and subepidermal skin when it was present on lesional skin. These findings suggest that the epidermal barrier integrity has been compromised and that FD has a persistent aberrant non-lesional skin microbiota (Lin, H.S. et. al., 2021). In 31% of cases, S aureus lacked both a superantigenic toxin and toxin specificity. Clinical improvement that was reached in most cases during medication was connected to the complete eradication of S aureus in all locations that were examined, a partial restoration of the normal microbiota, and a notable rise in the number of negative bacterial samples. This persistently out-of-balance sub-epidermal microbiota may serve as a reservoir for abnormal flora and explain why FD is chronic, opening new research directions for reestablishing healthy microbiota (Matard, B. et. al., 2020).

Several reports on the antibacterial properties of various plant extracts have been published. It is thought that medicinal plants are the finest place to get a wide range of medications. The medical plant *Hibiscus sabdariffa* is used to heal conditions like hypertension, coughing, biliousness, boils, and wounds in addition to providing delicious sustenance. Synthetic antibiotics are currently the major treatment for bacterial infections (Karimi, 2022). Yet, improper and excessive use of antibiotics has become a major contributor to the development of drug-resistant strains of various types of microbes, necessitating research into the antimicrobial capabilities of therapeutic plants (Nantumbwe, I., 2022).

Several health care systems are built around plants. Certain naturally occurring plant substances, such as anthraquinones, alkaloids, tannins, and cardiac glycosides, are manufactured for defense purposes. The *Hibiscus sabdariffa* is a member of the mallow family. The blossoms have historically been used as an anti-asthmatic (Quansah, L.,2021).

These antibacterial compounds have been discovered to exist in many plant parts through multiple examinations. The tropics and subtropics are home to more than 275 different species of hibiscus. *Hibiscus tiliaceus*. flowers are frequently utilized in birth control tablets and skin conditions. Several hibiscus species are utilized in traditional medicine for their leaves and blooms due to their antibacterial, anti-tyrosinase, and antioxidant properties. The cost, sensory impact, and side effects of using plant

extracts with antibiotics are reduced due to the reduction of minimum inhibitory concentrations (MICs) and synergistic action (Sweetline, C., 2020).

The aim of the research was to create a link between the natural solution's assumed realities and those of the modern scientific community. Furthermore, *Hibiscus sabdariffa's* ability to effectively inhibit the formation of bacteria.

Material and methods

It was an experimental study conducted in a lab using in vitro methods.

Obtaining Hibiscus Oil:

Infused hibiscus oil was purchased from Chiltan Pure in packaging of 250 mL.

ANTIBIOTIC SENSITIVITY TESTING

Antibiotic sensitivity testing (ABST) was used to find the best treatment against bacterial infection.

Solutions involving antibiotics for ABST.

Five milliliters of pure water were used to dilute ten milligrams of flucloxacillin. The liquid contained 2 mg/ml of flucloxacillin. Penicillin with 10,000 units of potency per milliliter and 40 micrograms of liquid gentamicin per milliliter.

Bacteria culture media preparation

Mueller Hinton Agar Media (MHA) was made by mixing 2.1 grams of bacteriological agar powder and 26.6 grams of Mueller Hinton agar powder in 700 milliliters of distilled water (Kanchan et al., 2019). The neck of the conical flask was sealed with aluminium foil to guard against contamination after it had been autoclaved and cooled to room temperature. After being cooled, the MHA medium was added to disposable culture plates that had been cleaned and sanitized. 0.4 g of autoclaved molten agar-agar were dissolved in 20 ml of distilled water at 120°C for 15 minutes. The mixture was then kept at 60°C in a water bath.

Controls and Microorganisms

Bacteria causing *foliculitis Staphylococcus* aureus was chosen because it is a commonly used strain for assessing the effectiveness of antibiotics and is regarded as a serious pathogen.

Microbial culture preparations according to standard

After 24 hours of sub culturing on sterile MHA plates, fresh cultures of S. aureus were produced. They were heated in an incubator to 370 degrees Celsius throughout the night. Little colonies were removed from the sterile wire loop and dissolved in 0.9% normal saline when the specified time for the incubation period had passed. By adding sterile normal saline, the bacterial solution's turbidity was brought into compliance with the 0.5 McFarland requirement. Repeating this procedure allowed the turbidity to reach the appropriate level. Four bacterial suspensions were created independently from each culture

collection and later labelled with the provenance of each (Khan *et. al.,* 2013)

Antimicrobial activity determination

Using ATCC cultures as the "standard microorganisms," sterile normal saline as the "negative control," and antibiotic solutions as the "positive controls," we tested for antimicrobial sensitivity using the agar well diffusion technique. To ensure uniform microbial growth, the inoculum was applied to the MHA plate using a sterile cotton swab in three distinct orientations. After then, the plate was turned by around 600 degrees. The agar surface was penetrated by five separate wells. The wells were 4 mm deep and 8 mm in diameter. To prevent leaks, a drop of melted agar was piped into each well bottom using a sterile plastic Pasteur pipette. To assure sterility, every measure was taken. Nobody managed to exit the wells.

- An aliquot of 75.0 mL hibiscus oil
- As gram-positive bacteria positive controls (S. aureus), solutions of flucloxacillin (75 l) and penicillin (75 l) were used.
- One negative control was 75 liters of sterile distilled water.

The plates were carefully labeled for quick and easy identification before being put in the incubator. After slowly diffusing sterile water, hibiscus oil, and antibiotic solutions for 15 minutes, the plates were covered with parafilm and incubated at 370C for 24 hours. After the plant extract, positive control, and negative control were incubated with the microbes, the widths of the inhibitory zones were measured for each bacterium. By employing the agar well diffusion approach, every zone of inhibition found was considered statistically significant. To determine the average inhibition zone for each isolate, the well diffusion experiment was performed three times (Peiris et al., 2019).

Minimum inhibitory concentration determination of hibiscus oil

The antibacterial activity of hibiscus oil might be measured according to Sanders' pour plate technique of obtaining the minimum inhibitory concentration (MIC) (Sander, 2012). Mueller Hinton agar powder (2.1g), a sterile component, was dissolved in 700ml of distilled water. Six milliliters of the 250 milligrams per milliliter concentration of the herbal combination were diluted to 125 milligrams per milliliter by adding six milliliters of distilled water to the mixture. The dilution series of the hibiscus oil formulation was obtained using the same methodology. The content of the hibiscus oil was then reduced from 250 mg/mL to 3.906 mg/mL. 3.0 ml of each doubling dilution (300 mg/ml, 150 mg/ml, and 75 mg/ml) were added and fully incorporated after 20 ml of molten MHA had been cooled to 500 C. Using sterile water, each concentration (31.25, 15.63, 7.81, and 3.91 mg/ml) was diluted before being put in dry culture plates. McFarland The inoculum was dissolved in 0.9% sodium chloride, or 0.5% standard normal saline. There were five-square grids on the backs of glass culture plates.

In each grid of the agar plate, PS. aureus and a sterile normal saline control were injected. For twelve hours, the plates were incubated at 37°C. After an overnight incubation, extracts were tested three times to find the minimum inhibitory concentration (MIC) (Gunasekara et al., 2017).

Statistical analysis

The mean and standard deviation were used to evaluate and report the data from the three runs of the experiment. Statistical analysis was performed using the SPSS (Statistical Program for the Social Sciences) version 21 tool.

Results

Hibiscus oil antimicrobial action

Significant antibacterial activity against S. aureus was demonstrated by the hibiscus oil. However, the hibiscus oil showed a lower zone of inhibition against the test germs (penicillin and flucloxacillin) as compared to the gold standard antibiotic. Strong inhibitory effectiveness of hibiscus oil was shown against *S. aureus*.

Table 1 Penicillin, Flucloxacillin, and Hibiscus oil values of microbes

Antimicrobial agent	*S. aureus*
Penicillin	51.23 ± 2.21
Flucloxacillin	48.54 ± 3.87
Hibiscus oil	46.91 ± 1.92

Minimum inhibitory concentration determination of hibiscus oil

The best concentration of hibiscus oil for killing microorganisms was found to be 250 mg/ml, according to the results. It was discovered that the growth of Staphylococcus aureus was inhibited by this concentration of hibiscus oil.

Table 2: Minimum inhibitory concentration determination of hibiscus oil

Concentration (mg/ml)	***Staphylococcus aureus***
300.00	ng
150.00	ng
75.000	ng
37.500	ng
18.750	g
9.3750	g
4.6875	g

Discussion

Much research is needed to establish a scientific basis for the effectiveness of medicinal plants, which have been used as natural remedies since ancient times and are seen as a supplement to manufactured medications. As a result, this study evaluated the well-known hibiscus oil's

microbiological resistance to conditions that affect the scalp and hair growth.

The results of this experiment showed that the antibacterial activity of hibiscus oil was only moderately efficient against gram-positive bacteria *Staphylococcus aureus* infection resulted in irritation of the hair follicles due to gram-positive bacteria.

A growing number of people are turning to medicinal plants as organic antibacterial agents. *Hibiscus sabdariffa* is frequently used to treat illnesses. The same outcomes were obtained from studies that demonstrated hibiscus oil's antibacterial efficacy against isolates of Escherichia coli O157:H7 from clinical, veterinary, and dietary sources. Ten grams of freeze-dried and crushed materials and one hundred milliliters of 80% aqueous methanol were used to extract the calyceal phenolics. The bactericidal efficacy of hibiscus methanolic extract was studied at dosages of 10%, 5%, and 2.5%. The inhibition zones were determined by measuring the amounts of inhibitory hibiscus that diffused into semisolid culture medium beneath the sorrel-impregnated disc and inhibited microbiological development. The results of this experiment showed that 10% of hibiscus was the most effective concentration, followed by 5% and finally 2.5%. For the hibiscus extract, the overall mean zone of inhibition was 12.66 mm for 10%, 10.75 mm for 5%, and 8.9 mm for 2.5%. The veterinary samples had the highest inhibitory zones (11.16 mm), whereas the food samples had the lowest (10.57 mm). The mean zones of inhibition from the nutritional, veterinary, and clinical sources varied significantly (P.05). Food sources showed the highest mean inhibition (15.370.61 mm), whereas clinical samples had the lowest (7.000.04 mm) based on the origin and sorrel extract concentration of the samples. At the end of the study, the antimicrobial activity was reported, and the antibacterial agent shown great potential by decreasing E. Coli O157:H7 at all levels (Fullerton et al., 2011).

The primary method of treating infections has been the use of synthetic antibiotics; however, over usage of antibiotics has resulted in the creation of strains of several groups of microbes that are multi-drug resistant. Another study that looked at the antibacterial capabilities of hibiscus extracts as well as the extracts' toxicological implications reported similar findings. In accordance with the least inhibitory concentration (MIC50) method, the antibacterial activity of ethanolic and aqueous extracts of hibiscus leaves and calyces was assessed. The brine prawn, Artemia salina, was also used to examine the toxicological effects based on LC50.The minimal inhibitory doses of the extracts were calculated using the statistical tool MINITAB 17 and the mortality rate of the nauplii was calculated using a simple percentage (P 0.05). Results from three replicates were reported as mean standard deviation. Hibiscus extract showed strong bacterial suppression. Compared to the leaves, calyces displayed greater toxicological effects and superior antibacterial

activity. Antimicrobial screening was successful with green hibiscus at the pre-flowering stage and red flowering. Hibiscus extracts had outstanding antibacterial and antifungal qualities. They can be researched to create new medications that can reduce the resistant strains. All the plant extracts had concentrations of more than 1000 mg/mL, indicating that brine prawn larvae were not hazardous to them (Salami, S.O. and Afolayan, A.J., 2020).

In a different study, an experimental comparison is made between the three hibiscus leaf extract extracts regarding the environmentally friendly synthesis of silver nanoparticles. The characterization procedure is then completed using an XRD, FTIR, AFM, UV spectrophotometer, and SEM investigation. The extract from hibiscus leaves was used to create silver nanoparticles that were between 25 and 50 nm in size. The extract from hibiscus leaves was used to create silver nanoparticles that were between 25 and 50 nm in size. Erythromycin became three times more effective after being applied to silver nanoparticles that had been treated with four distinct antibiotics. Recent research in the field of nanoparticle synthesis uses biological synthesis instead of chemical and physical methods to obtain non-toxic and uniformly sized nanoparticles; however, the microbial synthesis in biological synthesis requires sterile conditions and is time-consuming. These problems can be solved using green synthesis. Although relatively little work has been done utilizing hibiscus leaf extract, researchers are growing interested in the environmentally friendly manufacturing of silver nanoparticles from various plant extracts (Ukkund, S.J. et al., 2019).

The antibacterial, antifungal, and anticancer effects of *H. sabdariffa* water extract was examined in a different study. It provided the best inhibition zone, 35 mm, against *Escherichia coli* in antibacterial tests against those germs as well as *streptococcus mutans* and *Pseudomonas aeruginosa*. Moreover, *Fusarium graminearum* and *Rhizoctonia solani* were examined for antifungal efficacy, with R. solani showing the greatest growth suppression activity by 69%. In the anticancer activity MTT test against SR (lymphoma) and MCF7 (breast cancer) cell lines, the greatest cytotoxicity was found against MCF7, where it reached 64%. Due to its biological activity and global survivability, the *H. sabdariffa* water extract has intriguing qualities in the development of novel medications (Shamran, D.J. and Abed, E.H., 2020).

Therefore, hibiscus oil is an effective therapeutic since scientific evidence demonstrates the bioactivities of its ingredients, which include antibacterial, anti-inflammatory, analgesic, and cleansing properties. Furthermore, unlike antibiotics, bacteria do not develop resistance to this kind of natural remedy. Because they offer good value for the money, they are reasonably priced. Due of their accessibility and ease of preparation, plant materials are frequently used by those who employ natural therapies.

Conclusion

The antibacterial and antimicrobial properties of *H. sabdariffa* water extract was investigated in the current work. The findings of the studies suggest that the plant has a strong biological effect at high concentrations, while minor biological effects are seen at low quantities in several tests. To determine the precise mechanisms by which the effects of the plant extract were produced, more research will be conducted.

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