Characterization of Bioactive Chemical Compounds from *Staphylococcus aureus* and Evaluation of Antibacterial Activity

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**ABSTRACT**

**Aims and Objectives:** This research aimed to analyze the bioactive chemical products of *Staphylococcus aureus* and evaluate the antibacterial and in vitro antimicrobial activities of plant extracts against *Staphylococcus aureus*.

**Method:** The chemical components known as bioactives, which are sometimes referred to as secondary metabolites, were examined using gas chromatography-mass spectrometry (GC-MS) techniques. Subsequently, the antibacterial activity of the methanolic extract of *Staphylococcus aureus* was assessed in vitro.

**Results:** The GC-MS analysis of *Staphylococcus aureus* detected the presence of the following: The compounds listed include ethyl 12-aminododecanoate, 1,11-Diaminoundecan-6-ol, N-propylidenehydroxylamine, 3,5-diamo-2,6-dicyanopyrazine, and methyl 1-methylpiperidine-3-carboxylate. The compound is called 5-azoniaspiro[4,5]decane. The compounds mentioned include 3,3-Dimethyl-2-acetyloxirane, 2-Hexadecan, and 2-methyl-2-hexadecan. The compounds mentioned are 12,15-octadecadiynoic acid and 9,11-octadecadiynoic acid. 8-oxo-2-Ethyl-3,5-dimethylpyridine, 3,5-Dimethyl-2-ethylpyridine, ethyl 5,6-dimethylpyridine-3-carboxylate, 10-Oxododecanoic acid, and 12-Ethoxy-12-oxododecanoic acid. Glycyl-D-asparagine is a compound. 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-1H-pyrazin-2-one, and L-Leucyl-d-leucine. The evaluation of the antibacterial activity revealed that the metabolites of *Staphylococcus aureus* had a remarkably high level of activity against *Escherichia coli* (928±0.05). *Equisetum arvense* arvense extract exhibited significant antimicrobial activity against *Staphylococcus aureus*.

**KEYWORDS:** *Staphylococcus aureus*, Secondary metabolites, Antibacterial, GC/MS.

**INTRODUCTION**

*Staphylococcus aureus* is a type of bacteria that takes the form of a sphere and has a diameter of approximately 1 micrometer. Additionally, it is considered to be gram-positive. *Staph. aureus* creates colonies that are of a reasonable size and appear to be a "golden" color when they are grown on surfaces that are rich in nutrients. The capacity of *Staphylococcus aureus* to secrete toxins that cause damage to the membranes of host cells is one of its most important characteristics [1, 2]. Bacteremia induced by *Staphylococcus aureus* has been found to be responsible for more deaths than the entire number of deaths brought on by acquired immune deficiency syndrome (AIDS), tuberculosis, and viral hepatitis combined. Additional *Staph. aureus* infections, such as moderately severe skin infections including furuncles, abscesses, and wound infections, normally do not pose a threat to the patient's life, but they can inflict a significant amount of morbidity and suffering. Because of the high frequency with which they occur (several millions each year in the United States), they represent a substantial burden on the public's health.

The occurrence of systemic *Staphylococcus aureus* infection is consistently reliant on the penetration of germs through the protective layer of epithelial tissue. Minor abrasions on the skin can lead to the development of skin infections, which have the potential to become invasive. Nevertheless, *S. aureus* can also actively facilitate the formation of an opening in the epithelial layer. This is mostly because of α-toxin, which makes ADAM10 work and breaks down E-cadherin molecules. When people eat contaminated...
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foods that contain staphylococcal enterotoxins (SEs), S. aureus can cause food poisoning as a specific type of acute infection [4, 5].

Staphylococcus aureus is capable of inducing a range of skin and soft tissue infections as well as severe or potentially lethal conditions, including pneumonia, necrotizing fasciitis, and septicemia. Nosocomial Staphylococcus aureus infections impact various parts of the body including the bloodstream, skin, soft tissues, and lower respiratory tracts. Additionally, it can generate toxins that lead to toxin-mediated ailments such as toxic shock syndrome or food intoxications [6, 7]. Defining the genetic variables that influence whether a human's interaction with S. aureus leads to asymptomatic carriage or clinical illness is a challenging task.

Several bacterial pathogens have the potential to cause life-threatening illnesses. A Precise and expeditious diagnosis is crucial for the effective treatment of these contagious illnesses. Conventional methods for identifying microorganisms are laborious, necessitate specialised technology and experience [8]. Additional constraints of these methods include the high cost and limited accessibility of advanced microbiological equipment, as well as delays in transporting human specimens, such as fecal samples from patients with diarrhea, to the necessary laboratories. These factors continue to impede the timely implementation of appropriate curative measures in certain countries [9]. The aims of this study were to analyse the bioactive chemical compounds and assess their antibacterial properties.

MATERIALS AND METHODS
Optimal environmental conditions for growth and identification of metabolites

A strain of Staphylococcus aureus was isolated and subcultures were obtained on nutrient agar for 48 hours at a temperature of 22°C. The solution was subjected to incubation at a temperature of 4°C for a duration of 10 minutes, followed by agitation at a speed of 130 revolutions per minute for 10 minutes. The metabolites were isolated from the liquid culture and subjected to evaporation using a rotary evaporator at a temperature of 45°C [10, 11].

Performing a spectral study of the bioactive natural chemical components of Staphylococcus aureus utilizing (GC-MS)

The examination was carried out by employing a GC-MS technique with an Agilent 789 A device. The DB-5MS column from J&W Scientific in Folsom, California was utilized as the GC column. This column had the following properties: 30 m x 0.25 mm i.d. with a film thickness of 0.25 um. The temperature in the oven was maintained at the same level as in the previous investigation. Helium was used as the carrier gas, and the flow rate was set at one milliliter per minute. Through a transfer line that had been heated to 250 degrees Celsius, the effluent from the gas chromatography (GC) column was directly injected into the source of the mass spectrometer (MS). Ionization took place at a voltage of 70 electron volts (eV), and the temperature of the ion source was maintained at 230 degrees Celsius (°C). The measuring range encompassed 41 atomic mass units (amu) all the way up to 450.

Assessment of the antibacterial efficacy of secondary metabolite chemicals against three pathogenic bacteria.

Using a sterile cork-borer, wells with a diameter of five millimetres were created in the agar. Then, 25 μl of the sample solutions containing metabolites produced by Staphylococcus aureus were added to the wells. The test pathogens, namely E. coli, Proteus mirabilis, and Staph. Epidermidis, were collected using swabs and applied onto Muller Hinton agar plates [14]. Methanol served as the control solvent.

Antimicrobial efficacy of selected medicinal plant extracts against Staphylococcus aureus in a laboratory setting

Using a sterile cork-borer, wells with a diameter of five millimetres were cut from the agar. Then, 25 μl of the sample solutions of twelve medicinal plants were added to the wells. The plates were incubated for 48 h at room temperature. The antibacterial activity was assessed by measuring the diameter of the inhibitory zone seen after 48 hours of incubation. Methanol was employed as the control for the solvent. The reference antibacterial agents utilised were Rifambin and Cefotaxime [15]. The experiments were conducted in duplicate.

Statistical analysis

A number of statistical procedures, such as computing the mean value and carrying out an analysis of variance (ANOVA), were used to the examination of the data that had been collected from an SPSS (Version 11.6) database.

RESULTS and DISCUSSION

The GC-MS chromatogram displayed forty-five peaks corresponding to the identified chemicals. The compounds mentioned are ethyl 12-aminododecanoate, 1,11-Diaminoundecan-6-ol, and N-propyldenedehydroxylamine. Methyl 3,5-diamino-2,6-dicyanopyrazine The compound is known as 1-methylpiperidine-3-carboxylate. The compound is called 5-azaniaspiro[4.5]decan. The compounds listed are 3,3-Dimethyl-2-acetylxyorane, 2-Hexadecanol, and 2-methyl-2-hexadecanol. The compounds mentioned are 12,15-octadecadiynoic acid and 9,11-octadecadiynoic acid. 8-oxo The compounds mentioned are 2-Ethyl-3,5-dimethylpyridine, 3,5-Dimethyl-2-ethylpyridine, and ethyl 5,6-dimethylpyridine-3-carboxylate.<text>10-Oxododecanoic acid and 12-Ethoxy-12-oxododecanoic acid The compound is known as glycy[L-D-asparagine. The compounds mentioned include 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-1H-pyrazin-2-one, and 1-Leucyl-d-leucine. Piperazine-2,5-dione is a cyclic peptide where the hydrogen atoms at positions 2 and 5 are substituted by o xo</text>
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groups. It belongs to the class of 2,5-diketopiperazines and is a cyclic peptide. Flavacol is a naturally occurring substance present in Streptomyces and Aspergillus ochraceus, and there is documented information about it [16]. Leu-D-Leu is a dipeptide composed of L-leucine and D-leucine residues [17].

C14H29NO2
1-ethyl 12-aminododecanoate

C11H26N2O
1,11-Diaminoundecan-6-ol

C6H4N6
3,5-diamino-2,6-dicyanopyrazine

C8H15NO2
methyl 1-methylpiperidine-3-carboxylate

C6H10O2
3,3-Dimethyl-2-acetyloxirane

C17H36O
2-Hexadecanol, 2-methyl

C18H28O2
12,15-Octadecadiynoic acid

C9H18N+5-azoniaspiro[4.5]decane
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C12H20N2O
3,6-bis(2-methylpropyl)-1H-pyrazin-2-one

C12H24N2O3
l-Leucyl-d-leucine

The antibacterial efficacy of secondary metabolites produced by Staphylococcus aureus against three harmful microorganisms was investigated. The current study examined the bioactivity of the methanolic extract of Staphylococcus aureus and the standard antibiotics Rifambin and Cefotoxime against five tested pathogens: E. coli (19.28±0.08, 11.07±0.07, and 10.55±0.06), Proteus mirabilis (18.00±0.08, 09.00±0.05, and 12.09±0.07), and Staph. Epidermidis (18.05±0.08, 12.90±0.07, and 13.74±0.07). The metabolites of Staphylococcus aureus exhibited significant activity against Escherichia coli (9.28±0.05).

Figure 1. Metabolite products, Rifambin and Cefotoxime as anti-Bacterial activity against *Escherichia coli*

Figure 2. Metabolite products, Rifambin and Cefotoxime as anti-Bacterial activity against *Proteus mirabilis*
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Table 1. Displays the zone of inhibition (measured in millimetres) of various bioactive compounds and conventional antibiotics derived from plants against Staphylococcus aureus.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant extract</th>
<th>Diameter of zones of inhibition (mm) After 48 hr.</th>
<th>Mean Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Replicate 1</td>
<td>Replicate 2</td>
</tr>
<tr>
<td>1.</td>
<td>Artemisia annua (Crude)</td>
<td>10.32</td>
<td>10.50</td>
</tr>
<tr>
<td>2.</td>
<td>Quercus infectoria (Crude)</td>
<td>08.31</td>
<td>05.98</td>
</tr>
<tr>
<td>3.</td>
<td>Citrullus colocynthis (Crude)</td>
<td>05.15</td>
<td>06.74</td>
</tr>
<tr>
<td>4.</td>
<td>Althaea rosea (Crude)</td>
<td>13.00</td>
<td>12.09</td>
</tr>
<tr>
<td>5.</td>
<td>Coriandrum sativum (Crude)</td>
<td>09.22</td>
<td>09.75</td>
</tr>
<tr>
<td>6.</td>
<td>Melia azedarach (Crude)</td>
<td>10.13</td>
<td>10.00</td>
</tr>
<tr>
<td>7.</td>
<td>Origanum vulgare (Crude)</td>
<td>07.65</td>
<td>07.08</td>
</tr>
<tr>
<td>8.</td>
<td>Urtica dioica (Crude)</td>
<td>10.00</td>
<td>09.33</td>
</tr>
<tr>
<td>9.</td>
<td>Equisetum arvense (Crude)</td>
<td>13.05</td>
<td>13.00</td>
</tr>
<tr>
<td>10.</td>
<td>Foeniculum vulgare (Crude)</td>
<td>12.00</td>
<td>11.09</td>
</tr>
<tr>
<td>11.</td>
<td>Nigella sativa (Crude)</td>
<td>10.17</td>
<td>09.00</td>
</tr>
<tr>
<td>12.</td>
<td>Ocimum basilicum (Crude)</td>
<td>08.30</td>
<td>06.89</td>
</tr>
<tr>
<td>13.</td>
<td>Rifambin</td>
<td>09.50</td>
<td>11.00</td>
</tr>
<tr>
<td>14.</td>
<td>Cefotaxime</td>
<td>12.00</td>
<td>11.50</td>
</tr>
<tr>
<td>15.</td>
<td>Control</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

In vitro antimicrobial activity of plant extracts on Staphylococcus aureus

Diameter of zones of inhibition (mm) After 48 hr. for three repeated were (Artemisia annua (Crude) (10.32, 10.50 and 09.00 mm), Quercus infectoria (Crude) (08.31, 05.98 and 05.78 mm), Citrullus colocynthis (Crude) (05.15, 06.74 and 06.34 mm), Althaea rosea (Crude) (13.00, 12.09 and 12.85 mm), Coriandrum sativum (Crude) (09.22, 09.75 and 09.00 mm), Melia azedarach (Crude) (10.13, 10.00 and 10.87 mm), Origanum vulgare (Crude) (07.65, 07.08 and 06.99 mm), Urtica dioica (Crude) (10.00, 09.33 and 10.04mm), Equisetum arvense (Crude) (13.05, 13.00 and 12.09 mm), Foeniculum vulgare (Crude) (12.00, 11.09 and 12.04 mm), Nigella sativa (Crude) (10.17, 09.00 and 10.06), Ocimum basilicum (Crude) (08.30, 06.89 and 07.50 mm), and Equisetum arvense (Crude) (Crude) (12.71±0.18) was very highly active against Staphylococcus aureus Table 1.

The Staphylococci infection can vary in severity, ranging from a simple skin abscess or superficial tissue infection to potentially life-threatening illnesses [18]. According to global epidemiological statistics, Staphylococcus aureus...
biofilm often worsens skin and soft tissue infections (SSTIs),
causing them to become highly resistant to antibiotics. As a
result, the range of treatment options is significantly reduced
[19, 20]. The release of extracellular polymeric substance,
known as biofilm, reduces the effectiveness of treatment
drugs and promotes the growth of bacteria by means of
polysaccharide intercellular adhesin, a key element of
staphylococci biofilm [21]. The development of
Staphylococci biofilm, especially in wound infection and skin
abscess, typically intensifies the severity and likelihood of
bloodstream infection, thus contributing to the morbidity of
SSTIs mostly among hospitalised patients [22].
The plant extracts have antibacterial properties against multi-
drug resistant microorganisms. The findings strongly
support the idea that phytochemicals are a valuable source of
effective lead compounds for developing anti-staphylococci
drugs. The aqueous extract of these plants has demonstrated
potent anti-staphylococcal properties, which could be utilised
in the development of an antibacterial treatment for skin and
soft tissue infections (SSTIs) [23]. The application of skin
ointment, wound wash, antiseptics, and surgical wound
dressing pad can expedite the healing process by promoting
blood clotting, cell growth, collagen formation, wound
contraction, and reducing inflammation of infected skin [24].

CONCLUSION
The current research demonstrates that there are various
common household remedies that are easily accessible and
that have the potential to be utilized as alternatives to
conventional therapy as well as supplements to conventional
therapy. This knowledge will be of great use to developing
nations that lack adequate dental care facilities for their
citizens and have limited financial resources at their disposal.
According to the findings of the antibacterial activity test, the
metabolites produced by *Staphylococcus aureus* exhibited an
exceptionally high degree of activity against *Escherichia coli*
(9.280.05). *Staphylococcus aureus* was significantly
inhibited by the antibacterial activity of the *Equisetum
arvense* extract.

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