**Short Research Article**

**Antibacterial Activity of Lemongrass (*Cymbopogon citratus*) on *Streptococcus pyogenes* and *Staphylococcus aureus* Isolated from Throat of University Students.**

**Abstract**

Asymptomatic throat infections are on the increase among University students and a public health problem as pathogens can be transmitted from one person to another. The present study aims to determine the antibacterial activity of lemon grass (*Cymbopogon citratus*) against *Streptococcus pyogenes* and *Staphylococcus aureus* isolated from throats of University students. Samples of throat swabs were collected from thirty-three (33) students who were not showing any sign of illness at the time of collection. The bacterial species were isolated, identified and characterized using standard microbiological methods. Ethanolic and methanolic leaf extracts of lemongrass were tested against the isolates. Out of thirty-three throat swab samples screened, *Streptococcus pyogenes* was identified in 14(42.42%), including 8(47%) males and 6(37.6%) female samples. *Staphylococcus aureus* was detected in 12(36.36%), 5(29.4%) males and 7(43.75%) females. The inhibition zone diameter (IZD) of ethanolic and methanolic leaf extracts of lemongrass ranged from 6 mm – 25 mm and 4 mm- 22 mm respectively on the isolates. The minimum inhibitory concentrations (MIC) of the ethanolic and methanolic leaf extracts were 6.125 mg/ml and12.5 mg/ml respectively. Antibiotic susceptibility profiles revealed that *Streptococcus pyogenes* 6(42.9%) and *Staphylococcus aureus* 11(42.3%) were resistant to Azithromycin used as control antibiotic. The phytochemical analyses revealed the presence of saponins, tannins, flavonoids, terpenoids, steroids, oils and glycosides. In conclusion, lemongrass exhibited immense inhibitory action on the bacterial species. Thus, can be an alternative to antibiotics to reduce the multiplication and colonization of bacteria in the human throat.

**Key Words**: Lemon grass, Phytochemical, *Streptococcus pyogenes*, Antibacterial activity, Upper respiratory tract.

1. **INTRODUCTION**

Medicinal plantsand traditional medicine with antimicrobial activitie*s* havebeen usedwidely in the West African regions, and it is recognized in the primary healthcare system in many communities due to its affordability, low cost and accessibility [1]. Plants of medicinal importance have been shown to be effective even where treatments with antibiotics have failed [2]. Cymbopogon *citratus* is commonly known as lemon grass, barbed wire grass, citronella grass, fever grass [3]. It grows in sunny, warm, humid conditions of the tropics and grown in a wide variety of soil ranging from rich loamy to poor laterite soil but do not grow well in calcareous and water-logged soils [4]. *Cymbopogon citratus* belongs to Gramineae family which is rich in cyclic mono-terpene and other phytoconstituents like phenols, flavonoids, tannins and even alkaloids [5].These phytochemicals consist of quercetin, luteolin, apiginin, isoorientin 2’-o-rhamnoside and kaempferol that are known to have many benefits, especially in the fields of pharmacy, food, health and agriculture [6,7]. This plant also contains 1-2 percent essential oil on a wide variation of chemical composition as a function of habitat, genetic diversity and agronomic treatment of culture [8]. These secondary metabolites act on microorganisms and this opens to new avenues for novel natural antibiotics that can serve as substitutes for current antibiotics [9]. Some of these secondary metabolites possessed the ability to inhibit or kill different microorganism strains in unique mechanisms than the traditional antibiotics currently used that elated a significant clinical value in the treatment of resistant microbial strains even in corona virus [10]. The bioactivity of lemon grass have been extensively studied, especially as antioxidant, antimicrobial, antifungal, antibacterial, insecticidal and insect repellant activities [11].

*Streptococcus pyogenes* is a beta-hemolytic *Streptococcus* which is classified as lancefield Group A *Streptococcus* (GAS) [12]. It causes a wide range of infections in humans ranging from mild skin and upper respiratory tract infections to severe life-threatening conditions such as septicemia, pneumonia, necrotizing fasciitis and streptococcal toxic syndrome [12]. It remained asymptomatic in some individuals thereby allowing the transmission from one person to another. This could be as a result of immune system suppressing their multiplications. This organism is the most common bacterial cause of phargngitis among low income earners [13]. When screened and appropriately treated with antibiotics, pharyngeal carriers can be prevented from spreading respiratory infections in the community [12]. Unfortunately, these bacteria become resistant to commonly used antibiotics, hence, there is need to employ the use of affordable and available plant medicine. Group A streptococcus (GAS) elaborate several extracellular products which include streptolysin O(SLO) and derives its name from its oxygen lability [14].

Upper respiratory tract infection is caused by either viruses or bacteria. The common bacteria isolated from patients having throat infections are *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus* *spp*, *Klebsiella spp* and *Pseudomonas aeruginosa* [15]. The primary pathogen of orophargngitis is *Streptococcus pyogenes* where *Staphylococcus aureus* is a secondary pathogen. The sensitivity pattern of most of the beta-haemolytic organisms has shown an increasingly more resistant to the common and routine antibiotics, hence the reason for this study to provide an alternative means to treat resistant organisms. The aim of this study is to determine the antibacterial activity of *Cymbopogon citratus* (Lemon grass) on *Streptococcus pyogenes* and *Staphylococcus aureus* isolated from throats of University students.

1. **MATERIALS AND METHODS**
	1. **Study Design**

Experimental research design was used in this research work.

* 1. **Study Population**

A total of 33 students in the Department of Applied Microbiology of the University were used as the study population.

* 1. **Study Location**

This study was carried out at the Department of Applied Microbiology, Enugu State University of Science and Technology, Enugu State, Nigeria.

 **2.4 Collection of Plant Materials**

Fresh leaves of *Cymbopogon citratus* (Lemon grass) were collected from homes of people living at Agbani in Nkanu-West Local Government Area Enugu State, Nigeria. The leaves were identified in the Department of Applied Biology and Biotechnology, ESUT. These leaves were washed severally to remove dirt, dried in shade at room temperature, ground to powder and stored in a sterile air tight container.

**2.5 Preparation of extracts**

Fifty grams of *Cymbopogon citratus* (Lemon grass) leaf powder was soaked separately in 200 ml of ethanol and methanol respectively for 48 h and centrifuged at 3000 rpm(revolutions per minute) to enable proper diffusion of the active ingredients as described by [16]. After that, the contents were filtered using muslin cloth and filtered again using Whatman’s filter paper No 1. The filtrates were evaporated separately in a water bath and then used to check the antibacterial activities on *Streptococcus pyogenes* isolated from throat of University students.

**2.6 Sample Collection**

A total of thirty-three (33) throat swab samples were collected from both male and female University students. The students were not showing any sign of illness during the time of collection. The samples were labeled and taken to Department of Applied Microbiology laboratory of Enugu State University of Science and Technology (ESUT) and it was analyzed immediately.

**2.7 Sample Inoculation**

The throat swab sticks were inoculated into triplicates blood agar and mannitol salt agar plates and incubated at 370C for 24 h. The beta-hemolytic colonies and colonies from mannitol salt agar were sub-cultured in nutrient agar plates to obtain a pure culture. The pure colonies were transferred into nutrient agar slant for further tests.

**2.8 Characterization and Identification of isolates**

The isolates were identified based on the method described by [17].Gram stain and biochemical tests were carried out to identify the isolates. The biochemical tests are catalase, coagulase, oxidase, indole, motility test, citrate utilization and sugar fermentation tests.

**Indole Test**

Sterile test tubes containing 5 ml of tryptophan broth were set on a test tube rack, the tubes were inoculated aseptically and the bacteria growth added into it. The tubes were incubated at 37 ºC for 24 h. After 24 h, 0.5 ml of kovac’s reagent was added to it and allowed to stand for 5 minutes, formation of pink or red colour ring in the reagent layer on the medium (within 10 seconds) indicates positive result. Negative result shows no formation of pink or red colour ring.

**Citrate Test**

Simon citrate agar was prepared and sterilized into a test tube and slanted. It was allowed to solidify before organism was inoculated on the surface of the solidified Simon citrate agar in the test tube. It was covered with cotton wool and incubated at room temperature for 24 hours. For positive result, there will be visible growth and the medium will be blue while the negative result showed no visible growth and no colour change.

**Motility Test**

A small drop of bacterial suspension which was prepared in a broth was placed onto a cover slip. Petroleum jelly was applied on the edges of the coverslip and a cavity slide was inverted over the coverslip so that the drop was hanging inside the depression. The setup was flipped and examined under a light microscope (40x objective). Movements were observed. Active movement in different directions showed motility while no movement or only Brownian motion showed non-motility.

**Catalase Test**

Catalase test was done using a test tube; A clean test tube was placed on the rack, 1ml of hydrogen peroxide solution was poured into the test tube; Using a sterile glass rod, bacteria growth were picked from an agar plate and immerse it into the hydrogen peroxide solution. Presence of effervescence indicated catalase positive reaction whereas negative reaction showed no effervescence.

**Coagulase Test**

### **(Slide Test)**

About 10 µl of deionized water or physiological saline was added to a slide. Several colonies from a fresh culture were collected with an inoculating loop and were emulsified into the water to obtain a smooth milk-colored suspension. A drop of a rabbit or human plasma was added to the slide, and the clumping was observed immediately, not exceeding 10 seconds. A positive test was the demonstration of the agglutination of the bacterial cells after the plasma was added. A negative test was demonstrated by the lack of agglutination.

**Oxidase Test**

This was done using filter paper. A piece of filter paper was moistened with few drops of oxidase reagent. A sterile wire loop was used to pick a colony of the organism from the agar plate and was smeared onto the moistened filter paper. It was observed for a colour change within 10 – 30 seconds. Dark purple or blue colour developed within 10 seconds indicated a positive result while no colour change indicated a negative result.

**Sugar Fermentations**

A 10 ml of peptone water was introduced into 5 sterile test tubes respectively. Three (3) drops of methyl red was added into each of the test tubes, then Durham’s tubes were inserted in an inverted position into each of the tubes and sealed with foil before sterilization in an autoclave at 121 ºC for 10 minutes. One gram (1g) of respective carbohydrates: glucose, lactose, fructose, sucrose and mannitol, were added into 100 ml of sterile distill water and sterilized using membrane filter. A total of 1 ml of each of sterile sugar was added into each of the sterilized test tubes that contained the peptone water. Thereafter, the cultured organisms were inoculated into each of the tubes respectively. They were then incubated at 37 ºC for 24 h. Positive result indicates yellow colour while gas production were seen in the Durham’s tube.

**2.9 Determination of Antibacterial activity of *Cymbopogon citratus* (lemon grass)**

The antibacterial activity of *Cymbopogon citratus* (lemon grass) was determined by agar well diffusion method. A total of 1 g of each extract was dissolved in dimethyl sulfoxide (DMSO) to obtain a varying concentrations of 200,100, 50, 25, 12.5, 6.25 and 3.125 mg/ml as described by [18]. A standard inoculum of 1.5x108 cells which matched 0.5 McFarland standard was spread on the surface of sterile Muller Hinton agar plates in triplicates. A sterile 6 mm cork borer was used to make a hole on the Muller Hinton agar plates in which 0.1 ml of each of the plant extracts were added. The plates were incubated at 370C for 24 h. The antibacterial activity was detected by measuring zones of inhibition in millimetres. The least concentration of the extracts that inhibited the growth of bacteria was used as the minimum inhibitory concentration (MIC).Azithromycin antibiotic (200 mg/ml) was used as a control and zones of inhibition were determined.

**3.0 Determination of Qualitative components of the plant extract**

Qualitative analysis was carried out using standard protocol for determination of the following phytoconstituents: alkaloids, flavonoids, saponins, tannins, glycoside, reducing sugar, oils and steroids as described by [19].

The tests are as follows:

**Test for alkaloids:**

Five grams of the evaporated extract was boiled with 5 ml of dilute HCl in a water bath for 5 minutes. The mixture was cooled and filtered. And the filtrate was subjected to alkaloids test using Dragendoff’s reagent. The formation of a red precipitate indicates the presence of alkaloids.

**Test for flavonoids:**

Three grams of the extract was treated with ethyl acetate solution and heated in water bath for 1 minute. The mixture was cooled and filtered. The filtrate was shaken with 1% aluminium chloride solution and left for 10 minutes. The formation of yellow colouration indicates the presence of flavonoids.

**Test for saponins:**

One gram of the extract was boiled with 5 ml of distilled water for 5 minutes. The content was filtered while hot and the filtrate was treated with few drops of olive oil and vigorously shaken. The formation of emulsion indicates the presence of saponins.

**Test for tannins:**

Two grams of the evaporated extract was boiled with 45% ethanol for 5 minutes. The mixture was cooled and filtered. Then, the filtrates were then treated with few drops of lead acetate solution. The formation of gelatinous precipitate indicates the presence of tannins.

**Test for glycosides:**

One gram of the evaporated extract was boiled with 15 ml of distilled water for 5 minutes in a water bath. The mixture was cooled and filtered. Then, 0.2 ml of Fehling’s solution A and B was added to 5 ml of the filtrate and further boiled for 2 minutes in a water bath. The formation of a brick red colouration indicates the presence of glycosides.

**Test for reducing sugars:**

The plant extract was treated with Fehling’s solution (A and B) in a test tube. The colour change from deep blue to brick red indicates the presence of reducing sugar.

**Test for steroids:**

Two ml each of concentrated sulphuric acid (H2SO4) and acetic anhydride were poured into 5 ml each of the aqueous extract samples. A colour changed to violet indicates the presence of steroids.

**Test for Oils**:

About 0.2 g of the plant extract was pressed between filter papers and observed for transparency. A control was also prepared by placing 2 drops of olive oil on another filter paper and also observes for translucency. If the filter paper becomes transparent, it shows the presence of oils in the paste.

**3.1 Statistical Analysis**

All data collected were analysed using one-way ANOVA.

1. **RESULTS**

**Percentage (%) distribution of *Streptococcus pyogenes* and *Staphylococcus aureus* from throat of University students**

A total of thirty-three throat swabs were collected. It was observed that out of these samples, 14 (42.42%) of *Streptococcus pyogenes* and 12(36.36%) of *Staphylococcus aureus*were positives (Table 1).

**Table 1:** **Percentage (%) distribution of *Streptococcus* pyogenes and *Staphylococcus aureus* from throat of University students**

|  |  |  |  |
| --- | --- | --- | --- |
| Student | Number of throat swabs | Number positive for *Streptococcus pyogenes* | Number positive for*Staphylococcus aureus* |
| Male | 17 | 8(47.1) | 5(29.41) |
| Female | 16 | 6(37.6) | 7(43.75) |
| Total | 33 | 14(42.42) | 12(36.36) |

**Morphological and Biochemical Characteristics of the isolates**

The isolates were identified and the result is shown in Table 2.

Table 2: **Morphological and Biochemical Characteristics of the isolates**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Growth on blood agar** | **Gram reaction**  | **Catalase test** |  **Coagulase test** | **Oxidase test** | **Citrate utilization test** | **Motility test** | **Indole** | **Glucose fermentation** | **Lactose fermentation** | **Maltose fermentation** | **Fructose fermentation** | **Sucrose fermentation** | **Suspected organisms** |
| Dome-shaped with a smooth or moist surface and clear margins(β-hemolytic) | Positive cocci in chains | -ve   | -ve | -ve | -ve | -ve | -ve | A/G | A/G | A | A | A/G | *Streptococcus pyogenes* |
| Golden colonies with zones of clear beta-hemolysis | Positive cocci in butches | +ve | +ve | -ve | +ve | -ve | -ve | A/G | A/G | A/G | A/G | A/G | *Staphylococcus aureus* |

**Antibacterial activity of ethanolic and methanolic extracts of lemon grass on *Streptococcus pyogenes* and *Staphylococcus aureus* isolated from throat of University students**

The antibacterial activity of ethanolic and methanolic extracts of lemon grass was carried out on *Streptococcus pyogenes* and *Staphylococcus aureus*. It was observed that both extracts had appreciable activity on the test bacteria. The result is shown in table 3.

**Table 3: Zone of growth inhibition of ethanolic and methanolic extracts of lemon grass on *Streptococcus pyogenes* and *Staphylococcus aureus* isolated from throat of University students**

|  |  |  |
| --- | --- | --- |
| **Organism** | **Ethanolic** | **Methanolic** |
|  | **200** | **100** | **50** | **25** | **12.5** | **6.25** | **3.125** | **200** | **100** | **50** | **25** | **12.5** | **6.25** | **3.125** |
| *Streptococcus pyogenes* | **25** | **20** | **18** | **12** | **8** | **6** | **0** | **22** | **17** | **12** | **10** | **8** | **6** | **0** |
| *Staphylococcus aureus* | **20** | **18** | **15** | **13** | **10** | **0** | **0** | **18** | **14** | **10** | **7** | **5** | **4** | **0** |

**Minimum inhibitory concentration of Ethanolic and Methanolic Extracts of lemon grass on *Streptococcus pyogenes* and *Staphylococcus aureus* isolated from throat of University students**

The minimum inhibitory concentration of ethanolic and methanlic extracts of lemon grass on *Streptococcus pyogenes* and *Staphylococcus aureus*were evaluated. The result is shown in table 4.

**Table 4:** **Minimum inhibitory concentration of Ethanolic and Methanolic Extracts of lemon grass on *Streptococcus pyogenes* and *Staphylococcus aureus* isolated from throat of University students**

|  |  |
| --- | --- |
| Test bacteria  | Minimum inhibitory concentration (mg/ml) |
| **Ethanol** | **Methanol** |
| *Streptococcus pyogenes**Staphylococcus aureus* | 6.2512.5 | 6.256.25 |

**Antibiotic Susceptibility testing of the isolates to Azithromycin (200 mg/ml) as control antibiotic**

Azithromycin was used as a control antibiotic on these bacteria to ascertain their susceptibility profiles. It was found out that 8(57.1%) of *Streptococcus pyogenes* and 7(58.3%) of *Staphylococcus aureus* were sensitive to the antibiotic used (Table 5).

**Table 5:**  **Antibiotic Susceptibility testing of bacteria to Azithromycin**

|  |  |  |  |
| --- | --- | --- | --- |
| Isolates | Number of organism | Sensitive | Resistance |
|  |  |  |  |
| *Streptococcus pyogenes* | 14 | 8(57.1%) | 6(42.9%) |
| *Staphylococcus aureus* | 12 | 7(58.3%) | 5(41.7%) |
| Total | 26 | 15(57.7%) | 11(42.3%) |

**Phytochemical constituents of lemon grass**

The phytochemical constituents of lemon grass was determined and the result is shown in table 6.

**Table 6: Qualitative analysis of lemon grass**

|  |  |  |
| --- | --- | --- |
| Phytonutrients | Ethanol | Methanol Extracts |
| Flavonoids | + | + |
| Alkaloids | **+++** | **++** |
| Oils | **+** | **+** |
| Saponins | **+** | **++** |
| Tannins | **++** | **+** |
| Glycosides | **+++** | **+++** |
| Steroids | **++** | **\_** |
| Terprenoids | **+** | **++** |

**(+: presence; -: absence)**

1. **DISCUSSION**

The resistance of bacteria to commonly used antibiotics has become a public health problem. This has led to multidrug resistant bacteria being widely distributed in the environment, hence, there is need to employ an alternative route to solve this menace. The present study revealed that out of 33 swab samples collected, 14(42.42%) were *Streptococcus pyogenes* and 12(36.36%) were *Staphylococcus aureus* (Table 1). Also the present study showed that isolation rate of male was 8(47.1%) and 5(29.41%) for both *Streptococcus pyogenes* and *Staphylococcus aureus* respectively and female 6(37.6%) and 7(43.75%) respectively (Table 1). This study is in line with the work done by [18] who reported that out of 55 swab samples 37(67.27%) were positive. They also reported that 21(56.75%) were *Staphylococcus aureus* and 13(35.15%) were *Streptococcus pyogenes*. Also the isolation rates of male and female were 18(48.64%) and 19(51.38%) respectively. The reasons for the differences in the results among individuals could be as a result of immunity status and environmental factors. The present study revealed that the lemon grass had appreciable inhibitory effect on the test bacteria. The ethanolic extract ranged from 6-25 mm for *Streptococcus pyogenes* and 10-20 mm for *Staphylococcus aureus* while methanolic extract ranged from 6-22 mm for *Streptococcus pyogenes* and 4-18 mm for *Staphylococcus aureus* (Table 3). This study is in line with the work of [8] who had inhibition zone of 32 mm on *Staphylococcus aureus.* The ethanolic extract of lemon grass was found out to have more activity than the methanolic extract, this could be attributed to the phytochemical contents and the nature of extracting solvent [20]. This could also be due to the differences in their extraction efficiencies and the chemical properties of their active compounds. Hence, ethanol extracted more compounds responsible for the antimicrobial activity of the lemongrass than the methanol extract. This study also agrees with the work of [21] who had 53.1% of *Streptococcus spp* and 30.2% of *Staphylococcus spp* being sensitive to lemon grass oil. The antibacterial activity of lemon grass on these isolates could indicate that the extract has a wide spectrum of activity. The minimum inhibitory concentration in this present study varies from 6.25 mg/ml to 12.5 mg/ml (Table 4). This study is in contrast with the work of [21] who used 100 fold concentration of lemon grass oil to induce the antibacterial effect. The differences in the results could be as a result of concentration of extract used. It was found out that 8(57.1%) of *Streptococcus pyogenes* and 7(58.3%) of *Staphylococcus aureus* were sensitive to Azithromycin used (Table 5). This is in line with work of [8] who used Azithromycin at 200mg/5 ml against the isolates tested. The choice of Azithromycin as a control antibiotic is based on the fact that it is a broad-spectrum macrolide antibiotic with a long half-life and a high degree of tissue penetration [22] as well as used in the treatment of respiratory infections [23]. The phytochemical analyses revealed the presence of saponins, tannins, flavonoids, terpenoids, steroids, oils and glycosides (Table 6). This study is in tandem with the work of [8] who revealed the presence of flavonoids, tannins, saponins, steroids, terpenoids and coumarins. Balakrishnan *et al*. [24] in their work revealed the presence of tannins, saponins, flavonoids and phenols. The variations in the presence of these phytochemicals may be as a result of environmental factors like climate, altitude and rainfall [25]

1. **CONCLUSION**

Asymptomatic carriage rates of *Streptococcus pyogenes* and *Staphylococcus aureus* were high among university students. The carriage rate of *Streptococcus pyogenes* among males was higher than females and the carriage rate of *Staphylococcus aureus* among females was higher than males. Antibacterial activity of lemongrass inhibited the growth of these organisms, hence, it can be used as alternative to conventional antibiotics.

**ETHICAL APPROVAL**

Ethical approval was gotten from the ethical committee of the Faculty of Biological Sciences of the University.

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