***Original Research Article***

Evaluation of the antibacterial activity of aqueous and alcoholic extracts of Syzygium aromaticum against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pneumoniae

## Abstract

 Herbal medicinal products have been documented as a significant source for discovering new pharmaceutical molecules that have been used to treat serious diseases. Many plant species have been reported to have pharmacological activities attributable to their phytoconstituents such are glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes, etc. Syzygium aromaticum (clove) is a traditional spice that has been used for food preservation and possesses various pharmacological activities. S. aromaticum is rich in many phytochemicals as follows: sesquiterpenes, monoterpenes, hydrocarbon, and phenolic compounds. Eugenyl acetate, eugenol, and β-caryophyllene are the most significant phytochemicals in clove oil. Clove essential oil has biological activity relevant to human health, including antimicrobial, antioxidant, and insecticidal activity. This study was designed to determine the phytochemical constituents and antibacterial activity of aqueous and ethanolic extract of Syzygium aromaticum (clove) seed at varying concentrations; against Staphylococcus aureus, Escherichia coli, Streptococcus pneumonia, and Pseudomonas sp. Preliminary phytochemical screening of the S. aromaticum extracts was done using standard analytical methods. The aqueous and ethanol extracts of S. aromaticum were evaluated for antimicrobial activities against the isolates using agar well diffusion and broth dilution assay. The results of the phytochemical components revealed the presence of Alkaloids, flavonoids, Tannins, saponins, Glycosides, Terpenoids, and Phenol in the extracts. Ethanol extract of S. aromaticum displayed antibacterial activity against all the tested organisms with the highest activity (24mm at 100mg/ml concentration) on *Pseudomonas* sp. The aqueous extract of clove was found to be less active, though, it was active against all the organisms tested, with the highest activity on E. coli (2omm at 100mg/mL concentration). Both the aqueous and ethanolic extracts showed MIC at 6.25 mg/mL on all the tested isolates except the aqueous extract against E. coli which showed MIC at 12.5 mg/ml. MBC was only observed on ethanolic extract against Salmonella sp. and S. aureus both at 6.25 mg/ml. The results provide a scientific basis for the centuries-old traditional usage of S. aromaticum.

**keywords**: Syzygium aromaticum, Antibacterial activity, Phytochemicals, Agar well diffusion, aqueous extract ethanolic extract

**Introduction**: “Infectious disease is accounted as the world’s leading cause of premature death killing almost 50,000 people every day. Morbidity and mortality continue to be a major problem in many developing countries, especially among children. Infection that occurs due to many pathogenic bacteria such as *Escherichia* sp., *Staphylococcus* sp*.,* Strepyococcus sp., and *Salmonella* sp., are most common. Antibiotics normally treat infectious diseases and nosocomial infections. Antibiotics are substances produced by microorganisms or might be fully/ partly prepared by chemical synthesis. They inhibit the growth of microorganisms in minimal concentration. Antibiotics may be microbial origin or purely synthetic or semi-synthetic. They may either kill microorganisms outright or simply prevent their antimicrobial activity. They may inhibit cell wall synthesis, Protein synthesis, nucleic acid synthesis, enzymatic activity, and folate metabolism or damage cytoplasmic membrane” (Kapoor et al., 2017)

“Clove belongs to the tree *Eugenia caryophyllata* (Syzygium aromaticum) and is used as a spice in almost all the world’s fare. It has a very major role in the spice trade and is highly appreciated for its therapeutic properties. The oil of cloves has been used in a variety of health conditions including indigestion, generalized stress, parasitic infestations, cough, toothaches, headache, and blood impurities. The expert pane German Commission recently approved the use of its essential oil as a topical antiseptic and anesthetic. It has also been used for nausea and vomiting, while in tropical Asia, it has been given to treat such diverse infections as malaria, cholera, and tuberculosis. The main objective of the study is to evaluate the antibacterial activity of the essential oil of *S*. *aromaticum* against selected bacteria and to conduct phytochemical screening of the clove bud extract in ethanol solvents.The indiscriminate use of antibiotics has led to the emergence of new strains of microbial isolates. The ability of plants to synthesize compounds, such as phenols and alkaloids, through simple metabolic processes in their seeds, leaves, and roots, is a key element in their medicinal properties and their ability to suppress microbial activity”.) Habh.,et al.,2023)

## Materials and method:

*Syzygium aromaticum* (clove) is a traditional spice that has been used for food preservation and possesses various pharmacological activities. *S. aromaticum* is rich in many phytochemicals as follows: Sesquiterpenes, Monoterpenes, Hydrocarbon, and Phenolic Compounds. (Gaber El-Saber Batiha et al., 2020). In this study, ethanol extracts of *Syzygium aromaticum* (Bud) were tested against isolated bacteria. The isolates included gram-negative bacteria (*Escherichia Coli*, *Pseudomonas aeruginosa)* While gram-positive bacteria Included *(Staphylococcus aureus, Streptococcus pneumoniae).* The phytochemical careening of *Syzygium aromaticum (Bud)* extracts was performed using qualitative determination whilst the antimicrobial activity of ethanol extracts of bud was performed using the disc diffusion method.In the present study, cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella sp*. that create opportunistic infections were tested.

***Systematic classification:***

* ***Kingdom:Plantae***
* ***Division (or Phylum) :Tracheophytes***
* ***Class : : Magnoliopsida***
* ***Order : Myrtales***
* ***Family : Myrtaceae***
* ***Genus: Syzygium***
* ***Species : aromaticum***

 Picture 1 : fresh clove bud powder

In the present study, cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Salmonella sp*. that create opportunistic infections were tested.

***Escherichia coli***

***Escherichia coli*** is a [gram-negative](https://en.wikipedia.org/wiki/Gram-negative_bacteria), [facultatively anaerobic](https://en.wikipedia.org/wiki/Facultative_anaerobic_organism), [rod-shaped](https://en.wikipedia.org/wiki/Bacillus_%28shape%29), [coliform bacterium](https://en.wikipedia.org/wiki/Coliform_bacteria) of the genus [*Escherichia*](https://en.wikipedia.org/wiki/Escherichia) that is commonly found in the lower [intestine](https://en.wikipedia.org/wiki/Intestine) of [warm-blooded](https://en.wikipedia.org/wiki/Warm-blooded) organisms. “Most *E. coli* [strains](https://en.wikipedia.org/wiki/Strain_%28biology%29) are part of the normal [microbiota of the gut](https://en.wikipedia.org/wiki/Gut_microbiota), where they constitute about 0.1%, along with other [facultative anaerobes](https://en.wikipedia.org/wiki/Facultative_anaerobe).[[5]](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-pmid15831718-5) These bacteria are mostly harmless or even beneficial to humans.[[](https://en.wikipedia.org/wiki/Escherichia_coli#cite_note-Martinson-6) For example, some strains of *E. coli* benefit their hosts by producing [vitamin K2](https://en.wikipedia.org/wiki/Vitamin_K2). or by preventing the colonization of the intestine by harmful [pathogenic bacteria](https://en.wikipedia.org/wiki/Pathogenic_bacteria). These mutually beneficial relationships between *E. coli* and humans are a type of [mutualistic](https://en.wikipedia.org/wiki/Mutualism_%28biology%29) biological relationship—where both the humans and the *E. coli* are benefitting each other. *E. coli* is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under [aerobic conditions](https://en.wikipedia.org/wiki/Aerobic_organism) for three days, but its numbers decline slowly afterward” [3].

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***Pseudomonas aeruginosa***

***Pseudomonas aeruginosa*** is a common [encapsulated](https://en.wikipedia.org/wiki/Bacterial_capsule), [Gram-negative](https://en.wikipedia.org/wiki/Gram-negative_bacteria), [aerobic](https://en.wikipedia.org/wiki/Aerobic_organism)–[facultatively anaerobic](https://en.wikipedia.org/wiki/Facultative_anaerobe), [rod-shaped](https://en.wikipedia.org/wiki/Bacillus_%28shape%29) [bacterium](https://en.wikipedia.org/wiki/Bacteria) that can cause [disease](https://en.wikipedia.org/wiki/Disease) in plants and animals, including humans.[[1]](https://en.wikipedia.org/wiki/Pseudomonas_aeruginosa#cite_note-1)[[2]](https://en.wikipedia.org/wiki/Pseudomonas_aeruginosa#cite_note-2) “A [species](https://en.wikipedia.org/wiki/Species) of considerable medical importance, *P. aeruginosa* is a [multidrug-resistant](https://en.wikipedia.org/wiki/Multiple_drug_resistance) pathogen recognized for its ubiquity, its [intrinsically](https://en.wikipedia.org/wiki/Intrinsic_and_extrinsic_properties) advanced [antibiotic resistance](https://en.wikipedia.org/wiki/Antibiotic_resistance) mechanisms, and its association with serious illnesses – [hospital-acquired infections](https://en.wikipedia.org/wiki/Hospital-acquired_infections) such as [ventilator-associated pneumonia](https://en.wikipedia.org/wiki/Ventilator-associated_pneumonia) and various [sepsis](https://en.wikipedia.org/wiki/Sepsis) [syndromes](https://en.wikipedia.org/wiki/Syndromes). *P. aeruginosa* can selectively inhibit various antibiotics from penetrating its outer membrane - and has high resistance to several antibiotics. According to the [World Health Organization,](https://en.wikipedia.org/wiki/World_Health_Organization) *P. aeruginosa* poses one of the greatest threats to humans in terms of antibiotic resistance. The organism is considered [opportunistic](https://en.wikipedia.org/wiki/Opportunistic_infection) in so far as serious infection often occurs during existing [diseases](https://en.wikipedia.org/wiki/Disease) or conditions– most notably [cystic fibrosis](https://en.wikipedia.org/wiki/Cystic_fibrosis) and traumatic burns. It generally affects the [immunocompromised](https://en.wikipedia.org/wiki/Immunodeficiency) but can also infect the [immunocompetent](https://en.wikipedia.org/wiki/Immunocompetent) as in [hot tub folliculitis](https://en.wikipedia.org/wiki/Hot_tub_folliculitis). Treatment of *P. aeruginosa* infections can be difficult due to its natural antibiotic resistance” [2].

***Staphylococcus aureus***

**Staphylococcus aureus** is a [gram-positive](https://en.wikipedia.org/wiki/Gram-positive_bacteria) [spherically shaped](https://en.wikipedia.org/wiki/Coccus) [bacterium](https://en.wikipedia.org/wiki/Bacterium), a member of the [Bacillota](https://en.wikipedia.org/wiki/Bacillota%22%20%5Co%20%22Bacillota), and is a usual member of the [microbiota](https://en.wikipedia.org/wiki/Microbiota) of the body, frequently found in the [upper respiratory tract](https://en.wikipedia.org/wiki/Respiratory_tract) and on the [skin](https://en.wikipedia.org/wiki/Skin). It is often positive for [catalase](https://en.wikipedia.org/wiki/Catalase) and [nitrate reduction](https://en.wikipedia.org/wiki/Denitrification) and is a [facultative anaerobe](https://en.wikipedia.org/wiki/Facultative_anaerobic_organism), meaning that it can grow without oxygen. “Although S. aureus usually acts as a [commensal](https://en.wikipedia.org/wiki/Commensalism) of the human microbiota, it can also become an [opportunistic pathogen](https://en.wikipedia.org/wiki/Opportunistic_infection), being a common cause of [skin infections](https://en.wikipedia.org/wiki/Skin_infection) including [abscesses](https://en.wikipedia.org/wiki/Abscess#Classification), [respiratory infections](https://en.wikipedia.org/wiki/Respiratory_disease#Respiratory_tract_infections) such as [sinusitis](https://en.wikipedia.org/wiki/Sinusitis), and [food poisoning](https://en.wikipedia.org/wiki/Food_poisoning). Pathogenic strains often promote [infections](https://en.wikipedia.org/wiki/Infection) by producing [virulence factors](https://en.wikipedia.org/wiki/Virulence_factor) such as potent [protein](https://en.wikipedia.org/wiki/Protein) [toxins](https://en.wikipedia.org/wiki/Exotoxin), and the expression of a [cell-surface protein](https://en.wikipedia.org/wiki/Protein_A) that binds and inactivates [antibodies](https://en.wikipedia.org/wiki/Antibody). S. aureus is one of the leading pathogens for deaths associated with antimicrobial resistance and the emergence of [antibiotic-resistant](https://en.wikipedia.org/wiki/Antibiotic-resistant) strains, such as [methicillin-resistant S. aureus](https://en.wikipedia.org/wiki/Methicillin-resistant_Staphylococcus_aureus) (MRSA). The bacterium is a worldwide problem in [clinical medicine](https://en.wikipedia.org/wiki/Medicine#Clinical_practice). Despite much [research and development](https://en.wikipedia.org/wiki/Research_and_development), no [vaccine](https://en.wikipedia.org/wiki/Vaccine) for S. aureus has been approved” [1].

***Streptococcus pneumonia***

***Streptococcus pneumoniae***, or **pneumococcus**, is a [Gram-positive](https://en.wikipedia.org/wiki/Gram-positive), spherical bacteria, [alpha-hemolytic](https://en.wikipedia.org/wiki/Hemolysis_%28microbiology%29) member of the [genus](https://en.wikipedia.org/wiki/Genus) [*Streptococcus*](https://en.wikipedia.org/wiki/Streptococcus). *S. pneumoniae* cells are usually found in pairs ([diplococci](https://en.wikipedia.org/wiki/Diplococci)) do not form [spores](https://en.wikipedia.org/wiki/Bacterial_morphological_plasticity) and are non-motile. “As a significant human [pathogenic bacterium](https://en.wikipedia.org/wiki/Pathogenic_bacterium) *S. pneumoniae* was recognized as a major cause of [pneumonia](https://en.wikipedia.org/wiki/Pneumonia) in the late 19th century and is the subject of many [humoral immunity](https://en.wikipedia.org/wiki/Humoral_immunity) studies. *Streptococcus pneumoniae* resides asymptomatically in healthy carriers typically colonizing the respiratory tract, sinuses, and [nasal cavity](https://en.wikipedia.org/wiki/Nasopharynx). However, in susceptible individuals with [weaker immune systems](https://en.wikipedia.org/wiki/Immunocompromised), such as the elderly and young children, the bacterium may become [pathogenic](https://en.wikipedia.org/wiki/Pathogen) and spread to other locations to cause disease. It spreads by direct person-to-person contact via [respiratory droplets](https://en.wikipedia.org/wiki/Respiratory_droplet) and by auto-inoculation in persons carrying the bacteria in their upper respiratory tracts” [4].

**Plant collection and extraction**

The *S*. *aromaticum* seeds were shade-dried for a week and powdered using a laboratory blender. Extraction was performed by 25g of the powdered seed was weighed in a digital balance and soaked in 100ml ethanol. The conical flask with the solvent and the powered seed was covered with aluminum foil to prevent evaporation. The entire mixture was shaken at regular intervals to ensure thorough extraction. The stoppered container was allowed to stand for 24 hours at room temperature. After 24 hours the mixture was filtered and concentrated to obtain the desired extract. the extracts were transferred to weighing bottles to avoid contamination and used for further assays.

**Phytochemical analysis :**

Phytochemical screening is a systematic process used to identify and analyze the bioactive compounds present in plants. The procedure typically involves a series of tests to detect the presence of various classes of phytochemicals, such as alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids. The first step is often a preliminary examination, where the plant material is visually inspected for color changes or reactions indicative of specific compounds. Following this, specific tests are conducted for each class of phytochemical, utilizing reagents or solvents that react with or extract the target compounds. For example, Mayer’s and Dragendorff’s tests are commonly employed for alkaloids, while the ferric chloride test is used to detect the presence of phenolic compounds like flavonoids.

 Once the preliminary screening is complete, the identified phytochemicals can provide insights into the potential medicinal properties of the plant. Alkaloids, for instance, are known for their analgesic and anti-inflammatory effects, while flavonoids exhibit antioxidant properties. Tannins may contribute to the astringent qualities of certain plants, and saponins can have antimicrobial and expectorant properties. The results of phytochemical screening play a crucial role in guiding further research on the therapeutic applications of plants, contributing valuable information to the field of natural product pharmacology and drug discovery(Arshad M.S,2017).

The presence of various phytochemicals in the sample can be identified through qualitative tests:

1. **Steroids**: A reddish-brown ring formation after adding 0.5 ml of leaf extract, 2 ml of chloroform, and 1 ml of sulfuric acid indicates the presence of steroids. This reaction is associated with the Liebermann–Burchard test.
2. **Tannins**: The formation of a brownish-green color after adding 0.5 ml of extract to 2 ml of 0.1% ferric chloride solution confirms the presence of tannins. This reaction is a common qualitative test for tannins.
3. **Saponins**: The formation of foam after shaking a mixture of 2 ml of extract and 2 ml of distilled water for 10 minutes suggests the presence of saponins, which are known for their foaming characteristics.
4. **Flavonoids**: A yellow color appears upon adding 1 ml of 2N sodium hydroxide solution to 1 ml of the extract, indicating the presence of flavonoids.
5. **Quinones**: The test for quinones involves adding 1 ml of concentrated sulfuric acid to 1 ml of the extract. Further details on the outcome are needed for interpretation.
6. **Glycosides**: Mixing 2 ml of extract, 3 ml of chloroform, and 1 ml of 10% ammonium solution can reveal the presence of glycosides. Observing any color change or reaction is essential for interpretation.
7. **Cardiac Glycosides**: Adding 0.5 ml of extract, 2 ml of glacial acetic acid, and a few drops of 5% ferric chloride, followed by 1 ml of concentrated sulfuric acid, serves as a test for cardiac glycosides. Any observed changes should be noted for a conclusive interpretation.
8. **Terpenoids**: The addition of 0.5 ml of extract to 2 ml of chloroform, followed by the careful addition of concentrated sulfuric acid, serves as a test for terpenoids. Further observations are necessary for analysis.
9. **Phenols**: A blue or black color appears when 2 ml of distilled water and a few drops of 10% ferric chloride are added to 1 ml of the extract, indicating the presence of phenols.
10. **Proteins and Amino Acids**: The addition of a few drops of 0.2% Ninhydrin solution to 2 ml of extract and heating for 5 minutes results in a blue color, confirming the presence of proteins or amino acids.
11. **Anthraquinones**: A pink color appears when a few drops of 10% ammonia solution are added to 1 ml of the extract, indicating the presence of anthraquinones.
12. **Phlobatannins**: Mixing 1 ml of extract with 1 ml of 10% NaOH solution results in a yellow color, suggesting the presence of phlobatannins.
13. **Coumarins**: The presence of coumarins is confirmed by adding 1 ml of extract to 1 ml of 1N NaOH, placing the test tube in a boiling water bath for a few minutes, and shaking well. A yellow color indicates a positive result.

**Evaluation of antibacterial activity:**

 **Media employed:**

2.60 g of Mueller-Hinton agar was dissolved in 100ml distilled water and autoclaved at 121C for 20 minutes at 15 lbs pressure. The bacteriostatic property of the compounds was tested by agar well-diffusion method

**Preparation of antibacterial solution** :

The extracts were dissolved in sterile 1% DMSO. Streptomycin used as positive drug control was also dissolved in sterile 1% DMSO. The extracts were serially diluted to obtain concentrations of 10mg, 5 mg, 2.5 mg, 1.25 mg, and 0.625 mg per 50 for testing antibacterial activity. The compound that diffuses into the medium produces a concentration gradient. After the incubation period, the zones of inhibition were measured in mm.

**Test cultures**

The following common human pathogenic bacteria that are responsible for nosocomial infections were used for screening of antibacterial activity:

*Staphylococcus aureus*

*Escherichia coli*

*Psudomonas aurginosa*

*Streptococcus pneumonia*

**Swabs preparation**

A supply of cotton wool swabs on wooden applicator sticks was prepared to spread the culture. They were sterilized on paper in the autoclave.

## Experimental procedure

The antibacterial assay in the agar well-diffusion method was carried out by preparing plates with Mueller-Hinton agar medium for rapidly growing organisms. The medium in the plates was sterile and have a depth of about 4 mm. Pure culture were used as inoculums. The plates dipping a sterile swab into inoculums. The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60 C after each application. Finally the swab passed round the edge of the agar surface and allowed the inoculums to dry for 5-15 minutes with lid in place. Bores were ditched in plate. 50 1% DMSO dissolved antibacterial solutions were poured into the well. The plates were placed in an incubator 47C within 30 minutes of preparation for bacteria. After 24 hours incubation for bacteria, the diameter of zone (including the diameter disc) was measured. The measurements were taken with a ruler, from the bottom of the plate, without opening the lid. For each bacterial strain, negative controls were maintained where 1% DMSO was used instead of the extract.

**RESULTS**

## Phytochemical study

The phytochemical analysis of ethanol extract of *Syzygium aromaticum* seeds contains alkaloids, steroids, tannins, saponins, flavonoids, terpenoids, cardiac glycosides and phenolic compounds accompanied with a characteristic colour change but phbolatannins, aromatic acids, xanthoproteins were absent.

## Table 1: Phytochemicals in seed ethanol extract

|  |  |  |
| --- | --- | --- |
| S. No. | Compound | Extract |
| 1 | Alkaloids | + |
| 2 | Steroids | + |
| 3 | Tannins | + |
| 4 | Phbolatannins | - |
| 5 | Saponins | + |
| 6 | Flavonoids | + |
| 7 | Terpenoids | + |
| 8 | Cardiac glycosides | + |
| 9 | Phenolic Compound | + |
| 10 | Aromatic acids | - |
| 11 | Xanthoproteins | - |

 **(+: Presence, - : Absence)**

There are indeed various classes of chemicals found in plants, each with its own unique properties and functions. Steroids, tannins, saponins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, protection/amino acids, anthraquinones, flabatannins, and coumarins play different roles in plant physiology, defense mechanisms, and interactions with the environment. “The phytochemical screening of clove bud extracts has revealed a diverse array of bioactive compounds, providing valuable insights into the medicinal potential of these plant components. In the clove bud extract, the presence of steroids suggests potential anti-inflammatory and immune-modulating properties. Concurrently, the occurrence of tannins in both resin and seed extracts points to their antioxidant properties, though notably absent in the seed extract. Saponins, identified in the resin extract but lacking in the seed extract, are known for their role in various biological activities, including antimicrobial and anti-inflammatory effects. The absence of flavonoids in the resin extract and their exclusive presence in the seed extract suggests that the latter may hold antioxidative and anti-cancer properties attributed to flavonoids” [3].

## Antibacterial activity

The results show the different zone of inhibition between the different bacteria and clove buds. The obtained antibacterial activity shows different zone of inhibition against human pathogens. The zone of inhibition is tabled below.

**Table: 2 shows a Zone incubation formed by *S. aromaticum* seed ethanol extract**

**Concentration**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Bacteria** | **100****mg/mL** | **50****Mg/mL** | **25****Mg/mL** | **12.5****Mg/mL** | **6.25****Mg/mL** | **Positive** |
| ***E. coli*** | 20mm | 15mm | 10mm | 7mm | 8mm | 25mm |
| ***Staphylococcus aureus*** | 24mm | 18mm | 14mm | 10mm | 10mm | 25mm |
| ***Streptococcus******pneumonia*** | 21mm | 17mm | 13mm | 8mm | 7mm | 30mm |
| ***Pseudomonas* sp.** | 25mm | 17mm | 13mm | 8mm | 8mm | 25mm |

**Figure: 1 Graphical representation of the activity of Clove bud against Human Pathogens**

35

30

25

20

15

10

5

100 mg/ml

50 mg/ml

25 mg/ml

12.5 mg/ml

6.25 mg/ml Positive

0

Escherchia coli

Staphylococcus

aureus

Streptococcus

pneumoniae

Pseudomonas

aeruginosa

**Bactria**

 In a recent study, the highest antibacterial activity was observed in clove bud dissolved in ethanol solvent. This particular combination exhibited an impressive 24 mm zone of inhibition against the bacteria Staphylococcus aureus, indicating its potent antimicrobial properties. Notably, streptococcus pneumonia demonstrated the highest resistance with a zone of inhibition measuring 21mm highlighting the varying susceptibility of different bacteria to based on this solution. Additionally, Pseudomonas displayed aeruginosa resistance level of 25 mm of the zone of inhibition. The overall findings suggest that the clove in ethanol solvent holds promising antibacterial efficacy, showcasing its potential as an effective agent against a range of bacterial strains. “It's worth noting that the positive control, likely representing a standard antibacterial agent, exhibited the maximum average resistance. This emphasizes the remarkable antibacterial activity of the bud in comparison, reinforcing its significance in the context of combating bacterial infections. The consistent high-performance results against various bacteria underscore the potential practical applications based on this solution in the development of antimicrobial agents or medical interventions “[5].

**Figure : 2 Phytochemical screening of ethanol extract**





## Figure 3 : Representation of antibacterial activities of clove bud



**Figure: 4-Antibacterial activity against *Escherichia coli***



**Figure: 5** **-Antibacterial activity against *staphylococcus aureus***

 **Figure: 6. Antibacterial activity against *Pseudomonas aeruginosa***



**Figure:7. Antibacterial activity against Streptococcus pneumoniae**



**Discussion**

“The results of the present study suggested that several phytochemicals are present in Syzygium *aromaticum* bud extracts. The presence of the phytochemicals can be correlated with the fact that solvent extracts showed antibacterial activity against the bacterial strains. Phytochemicals give plants their color, flavor, and smell and are part of a plant’s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites. The phytochemicals alkaloid, terpenoids, flavonoids, steroids, saponin and tannin were present in *Syzygium aromaticum* extracts according to this study (Table:1) and (Figure: 1). The results are by the findings of other authors who have studied this plant” (Teles, A.M 2021).

According to this study, alkaloid is present in the extracts. Alkaloids comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents, anesthetics, and Central Nervous Stimulants. Alkaloids are known to play some metabolic roles and control development in living system. It also interferes with cell division, hence the presence of alkaloids in clove could account for their use as antimicrobial agents.

Aboaba, et al. (2017) reported that “the antimicrobial properties of substances are desirable tools in food spoilage and food safety. This suggests that the *Syzygium aromaticum* extracts which have been confirmed to contain alkaloids may also be useful as preservatives in food. Terpenoids are useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, and- hyperglycemic, anti-inflammatory, and simmunomodulatory properties. Flavonoids are also present in the extracts as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity”.

The results of the antibacterial activity of Syzygium *aromaticum* extracts against human pathogens show (Figures; 2, 3, 4, and 5) that the ethanol extracts are more effective against the tested isolates than aqueous extracts. *E*. *coli* and *Streptococcus* were also more susceptible to the extracts in comparison respectively. The result of the antimicrobial activity of Syzygiumaromaticumin this study conformed with the study conducted by many researchers. The extract of *Syzygium aromaticum* showed the highest zone of inhibition against *Streptococcus spp*. And lowest zone of inhibition against *E*. *coli*. *Syzygium aromaticum* extract also showed a lower zone of inhibition against *Staphylococcus* compared to the Gram-negative bacteria. Based on the susceptibility of the organisms to the extracts, *E*. *Coli* was found to be the highest susceptible organism with an average zone of inhibition followed by *Staphylococcus spp*, *S*. *aureus*, and *Pseudomonas* with the least average zone of inhibition. The different concentration of zones of inhibition is shown in (Figure: 6).

The antibacterial activities of the extracts are expected due to the presence of compounds such as alkaloids, flavonoids, and tannins. The results obtained in this study collaborate with the report of Nzeako, et al. (2016) which found that “clove extract possessed a broad spectrum of antimicrobial activity exhibited for both bacteria and fungi *S*. *aureus*, *E*. *coli*, *P*. *aeruginosa* as well as against *S*. *pyogenes*, *Corynebacterium*, *Salmonella*, *Bacteroides* and *C*. *albicans* at various dilutions of the extracts”. The result of this justified that of Sofia et al (2007), who tested “the antimicrobial activity of different Indian spice plants such as mint, cinnamon, mustard, ginger, garlic, and clove, the result showed a complete bactericidal effect against all the human pathogens tested *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus cereus* was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action”.

Conclusion:

To conclude, this study revealed that *Syzygium aromaticum* extracts possess medicinal properties and antibacterial activity that inhibit bacterial growth. The present study shows that *Syzygium aromaticum* ethanol extracts are more effective against all tested bacterial strains than aqueous extracts. *E*. *coli* and *P*. *aeruginosa* were also more susceptible to the extracts while *P*. *aeruginosa* was the least susceptible. The antibacterial activities of the extracts are expected perhaps due to the presence of bioactive compounds. The results of the present study have justified the therapeutic potential of *Syzygium aromaticum* and used as a dietary supplement for food preservation in addition to pharmacological values. Phytochemical screening unveiled the presence of steroids, tannins, saponins, flavonoids, quinones, cardiac glycosides, terpenoids, and coumarins in the resin and seed extracts. To understand the antimicrobial properties and chemical composition of *Syzygium aromaticum*, supporting its potential applications in pharmaceuticals and therapeutic interventions.

Disclaimer (Artificial intelligence)

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Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Details of the AI usage are given below:

1.

2.

3.

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