***Original Research Article***

**PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *Syzygiumaromaticum* ETHANOL EXTRACT ON *Escherchia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae***

## Abstract

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, flavanoids, 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.

**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.

## 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 (*Escherchia 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.

***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***

E. coli is a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia coli* that is commonly found in the lower intestine of warm-blooded organisms. Most *E*. *coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination. *E*. *coli* and other facultative anaerobes constitute about 0.1% of gut microbiota and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia uremic syndrome, however, healthy individuals of all ages are at risk of severe consequences that may rise as a result of being infected with *E*. *coli.*

***Pseudomonas aeruginosa***

*P*. *aeruginosa* is a common encapsulated, Gram-negative, rod-shaped bacterium that can cause disease in humans. A species of considerable medical importance, P. aeruginosa is a multidrug-resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanism, and its association with serious illnesses hospital-acquired infections such as ventilator-associated pneumonia, and various sepsis syndromes. The organism is considered opportunistic in so far as serious infection often occurs during existing diseases or conditions- most notably cystic fibrosis and traumatic burns. In animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis. If such colonizations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal. *P*. *aeruginosa* typically infects the airway, urinary tract, burns, and wounds and also causes other blood infections.

***Staphylococcus aureus***

*Staphylococcus aureus* is a Gram-positive, rounded-shaped bacterium that is a member of the Firmicutes, and it is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. An estimated 20% to 30%A of the human population are long-term carriers of *S*. *aureus* which can be found as part of the normal skin flora, in the nostrils, and as a normal inhabitant of the lower reproductive tract of women. S. *aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boil, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. It is still one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery. Each year, around 500,000 patients in hospitals of the United States contract a staphylococcal infection, chiefly by S. aureus. Up to 50,000 deaths each year in the USA are linked with S. aureus infections.

***Streptococcus pneumonia***

*Streptococcus pneumoniae* Is a Gram-positive, Lancet-shaped bacterium and a cause of community-acquired Pneumonia. Pneumococcal Infections are present throughout the World and Are Most prevalent during the winter and early spring months.

**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)

 Phytochemical screening of moringa involves identifying and analyzing bioactive compounds present in the plant. Commonly found phytochemicals in moringa include flavonoids, alkaloids, saponins, tannins, and terpenoids. Various tests like the alkaloid Dragendorff’s, froth test for saponins, and ferric chloride test for flavonoids can be used to detect these compounds (Rohela *et al.,* 2016).

The reddish-brown ring formed after adding 0.5 ml of leaf extract, 2 ml of chloroform, and 1 ml of sulfuric acid suggests the presence of steroids in the sample. This reaction is often associated with the Liebermann–Burchard test for steroids. The formation of a brownish-green color after adding 0.5 ml of extract and 2 ml of 0.1% Ferric chloride solution indicates the presence of tannins in the sample. This reaction is commonly used in the qualitative analysis of tannins. 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 in the sample. This foaming characteristic is a common test for the presence of saponins in plant extracts Adding 1 ml of 2N sodium hydroxide solution to 1 ml of the extract results in a yellow color, indicating the presence of flavonoids. Upon adding 1 ml of concentrated sulfuric acid to 1 ml of the extract, the test for quinones is performed. Further details on the outcome are needed for interpretation. Mixing 2 ml of extract, 3 ml of chloroform, and 1 ml of 10% ammonium solution can reveal the presence of glycosides. Additional observations or color changes are necessary for a complete interpretation. By 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, the test for cardiac glycosides is conducted. Details on any observed changes are required for a conclusive interpretation. The addition of 0.5 ml of the extract to 2 ml of chloroform, followed by the careful addition of concentrated sulfuric acid, is a test for terpenoids. Further details on the results are necessary for analysis. After adding 2 ml of distilled water and a few drops of 10% ferric chloride to 1 ml of the extract, a blue or black color suggests the presence of phenols. After adding a few drops of 0.2% Ninhydrin solution to 2 ml of the extract and heating for 5 minutes, the appearance of a blue color indicates the presence of proteins or amino acids. The addition of a few drops of 10% ammonia solution to 1 ml of the extract results in a pink color, indicating the presence of anthraquinones. Mixing 1 ml of extract with 1 ml of 10% NaOH solution leads to a yellow color, suggesting the presence of phlobatannins. 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, the appearance of a yellow color Indicates the presence of coumarins.

**Evaluation of antibacterial activity:**

 **Media employed:**

2.60 g of Mueller-Hinton agar was dissolved in 100ml distilled of 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 | Flsvonoids | + |
| 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.

## 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.

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





**Figure : 3 -Antibacterial activity against *Streptococcus pneumonia***

## Figure 4 : Representation of antibacterial activities of clove bud



**Figure: 5-Antibacterial activity against *Escherichia coli***



**Figure: 6** **-Antibacterial activity against *staphylococcus aureus***

 **Figure: 7 Antibacterial activity against *Pseudomonas aeruginosa***



**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, flavour, 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, anaesthetics, 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 immunomodulatory 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 *Syzygium aromaticum* in 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.

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