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Antibacterial effect of aqueous extract of *Syzygium Aromaticum* and floxacin against gram negative and positive bacteria: an *in-vitro* study

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Abstract

Syzygium aromaticum is an antibacterial activity against various pathogenic microorganisms. A broad range of anti-disease activities estimate its potential therapeutic uses in treating numerous infectious disorders. The study aims to understand better how Syzygium aromaticum extract inhibits human pathogenic bacteria and demonstrate how the extract works to prevent the formation of bacterial biofilms and adhesion. The antibacterial action of the aqueous extract of Syzygium aromaticum was evaluated by using disc diffusion and the assay Agar-well diffusion. Its efficacy was compared with the antibiotic and determined. Additionally, tests on adherence and biofilm formation were conducted. All bacteria isolated from gram-negative (G -ve) and gram-positive (G +ve) bacteria were sensitive to *Syzygium aromaticum* extract and the range of inhibition zone (20 to 28) mm. Most isolated bacteria were sensitive to floxacin. Most bacterial isolates of Gram-negative bacteria exhibited Moderate adherence and biofilm activity to these extracts. Some bacteria isolates exhibited high adherence and biofilm activity to aquatic extracts of *Syzygium aromaticum*. The studys' findings were that the extracts from *Syzygium aromaticum* were highly effective against a variety of G-positive and G-negative isolated clinically, suggesting that they are superior to antibiotics sold in stores. Apart from strong resistance to adhesion and biofilm development.

Keywords

antimicrobial properties; *Syzygium aromaticum*; floxacin; biofilm formation; adherence inhibition

Introduction

Around the world, herbal medicine is becoming a more popular alternative therapy. As a result, herbal remedies are becoming more prevalent in medicine. Herbal extracts work because they bind to specific chemical receptors in the body. Herbal medicines are safer to use than conventional prescriptions and have less adverse effects when compared to traditional medications (1). *Syzygium aromaticum* is a Scientific name of clove which is aromatic flower buds of an Indonesian native tree belonging to the Myrtaceae family. The bioactive substances in cloves, including caryophyllene, eugenol and acetyl eugenol) have been found to have numerous physiological properties, including antioxidant, antimicrobial, analgesic, anti-inflammatory, anticancer and anaesthetic effects (2). All phytochemical molecules that were separated from *S. aromaticum*. Eugenol, cinnamaldehyde, carvacrol and thymol are the main constituents extracted from the clove essential oil. The active ingredient in clove essential oil is eugenol, which the FDA generally regards as harmless (3). Because of its physiological properties, clove essential oil is helpful in the industrial pharmacy, active packaging, biomedical, cosmetics, food and hygienic industries. Clove essential oil is often used in cooking as a natural colouring agent, spice and preservative (4).

The most important chemical compounds of clove are phenolic substances such as hydroxyphenyl propionate, hydroxybenzoic acids, hydroxycinnamic acids and flavonoids, the richest source of phenolic compounds is phenylpropanoids like eugenol, approximately 50 - 88 %, depending on the source of the plants that is the principal constituent of clove oil, widely used by dentist as anaesthetic and analgesic (toothache) drug. Eugenol has a strong cardiovascular, anti-inflammatory and antioxidant quality in addition to analgesic and local anaesthetic effects (6). A commercial sample of clove oil contains approximately 70 % eugenol and 15 % β-caryophyllene. Clove oil's eugenol content varies according to the distillation method, location, soil type and climate (7). It has been suggested that clove essential oil might be contained in films, microcapsules, nanocapsules, microparticles and nanocomposite materials. These techniques enhance the stability of clove essential oil in aqueous media, which raises its bioavailability, decreases its side effects, allows the encapsulated substance to release gradually, shields it from the environment, or covers up its overpowering smell. Nanocarriers and microcarriers with designer characteristics are especially attractive due to their commensurate increases in reactivity and excellent surface-to-volume ratios (8). The chemical composition of clove essential oil is influenced by the clove's age, geographical origin, developmental stage and postharvest handling (drying and storage). Eugenol is abundant in clove buds (the quantity is forty-nine times greater than in Genovese basil).

The young buds contain a lower concentration of eugenol than mature buds and leaves because they are converted by alcohol acyltransferase of eugenol into eugenol acetate (9). Clove aqueous extract at 3 % and 1 % exhibited a complete bactericidal effect against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* (10). According to the component analysis of essential oils, temperature and humidity can impact the aroma's composition, leading to the generation or degradation of specific components (11). Molecular mechanisms of eugenol as antimicrobial activity induce cell lysis in G-positive & G-negative bacteria by Inhibition of ERK, IKK/NF-κB and p38MAPK signalling pathways (12). It might function by rupturing the cytoplasmic membrane, which raises nonspecific permeability in the membrane and influences ATP and ion transport (13). Initially, eugenol changes the permeability of the membrane; this hyper-permeability is followed by ion leakage and a significant loss of other cellular components, which finally leads to cell death. Additionally, eugenol changed the fatty acid composition of certain bacteria's cell membranes (14).

The mechanism by which eugenol causes intracellular content leakage of bacteria by disrupting the lipid component of the membrane. The total surface of the bacterial membrane and the composition of the lipid content may further vary the strength of the effect. Modification of cell permeability may permit the oil to interact more with the intracellular sites, causing cell destruction (15). The objective of the investigation is to elucidate the inhibitor effect of *Syzygium aromaticum* extract against humans' pathogenic microorganisms that, demonstrating how the mechanism of action of the extract against the forming of biofilm and bacterial adherence.

Materials and Methods

The preparation of aqueous extract from *Syzygium aromaticum* (25 %) concentration and floxacin according to Hindi NK of aquatic garlic extract (16).

Bacterial and Isolates

Twenty-two specimens of isolated bacteria were used in the study. Isolated bacteria activate thrice continuously on nutrient agar, then stored at 4°C as slanted nutrient agar. The isolated bacteria were documented by various biochemical tests (17). The fourteen (G-ve) bacteria were *Aggregatibacter actinomycetemcomitans, Prevotella intermedi, Porphyromonas gingivalis, Pseudomonas flourscences, Pseudomonas aeroginosa, Escherichia coli, Proteus merabilis, Proteus vulgaris, Acinetobacter, Enterobacter aerugenes, Klebsiella pneumonia, Serratia spp.,Salmonella typhi* and *Salmonella typhimurum* The eight (G+ve) bacteria were *Staphylococcus saprophyticus, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus mutanus, Streptococcus pneumonia, Streptococcus pyogenes, Streptococcus aglagtia and Streptococcus faecalis.*

Antimicrobial activity test by agar-well diffusion assay (*In vitro*)

This was analyzed using the standard protocol for detecting antimicrobial activity (18).

Antimicrobial activity assay

According to Forbes biochemical tests, antimicrobial activity was detected by agar-disc diffused for antibiotics (17). The test was achieved in triplication.

Adherence test

Bacterial adherence to the human cell's epithelial membrane is one of these microorganisms' chief and significant virulence factors. It could be recognized using the technique for gram-negative only (19,20).

Formation of Biofilm Assay:

The assay of the tissue culture plate method (TCP) or semi-quantitative micro-titer plate test as per standard protocol the forming of biofilm (for G-negative only) is given in Table 1 (21).

Table 1. Adherence of microorganism and biofilm forming by TCP method (22)

Mean of optic density value at 630 nm	Adherence	Formation of biofilm
0.120<	non	non
0.240 - 0.120	moderately	moderate
> 0.240	strong	high

Results and Discussion

Antibacterial effect of *Syzygium aromaticum* and floxacine

The antibacterial effect of *Syzygium aromaticum* at 25% concentration against bacteria by agar well method was studied (Fig. 1-2). All bacteria isolated from G-negative and G-positive bacteria were sensitive to this extract and range of inhibition zone (20 to 28) m. The antimicrobial effect of floxacine against G-negative and G-positive bacteria by disc diffusion method (Fig. 3-4), most bacterial isolated of (G-ve) and (G+ve) bacteria were resistant to this antibiotic. The issue of antibiotic resistance phenomena rises in human infections and becomes fatal. This represents a considerable problem in the medical field, leading to the necessary discovery and use of novel molecules, like *Syzygium aromaticum* extract (23). This extract has lipophilic properties and acts on different cellular targets. Furthermore, they can interfere with proteins and enzymes' function and metabolism of fatty acids on other cellular targets (24). Clove essential oil has been demonstrated to have antimicrobial activity with a broad-spectrum inhibitory activity against pathogens. The primary chemical composition's ortho and meta locations, respectively, these function groups interact with the cytoplasm membrane of bacterial cells (25). Due to its lipophilic properties, clove essential oil can penetrate cell membranes. Clove essential oil interferes with the activity of the proton pump, causes cellular membrane deterioration, leaks contents of the cell and ultimately results in cell death (26).

Clove essential oil can inhibit G-negative bacteria (*Agrobacterium, E. coli, Erwinia carotovora, Salmonella, Pseudomonas aeruginosa*) and Klebsiella pneumonia G-positive bacteria (*L. monocytogenes, S. aureus and Streptococcus*), *Aspergillus (A. ochraceus, A. flavus and A. parasiticus) C. albicans, Penicillium* and yeast (27). Clove Essential Oil inhibits G-positive bacteria largely than G-negative bacteria. This is explained by the fact that G-positive bacteria have a diffusible mucopeptide coating that makes them susceptible to antibiotics. On the other hand, the diffusion rate of lipophilic antibacterial chemicals through the cell membrane of G-negative bacteria can be considerably decreased by the complex layer of lipopolysaccharide present in their outer cell membrane (28). Likewise, food-related pathogens have shown greater sensitivity to clove essential oil than probiotics and fungi (29). On the other hand, ofloxacin is a fluoroquinolone antibiotic whose primary mechanism of action is inhibiting the DNA gyrase of bacteria. It is not very active against anaerobes but exhibits a wide range of activity against aerobic (G-ve) and (G+ve) bacteria when tested *in vitro*. The extracts of *Syzygium aromaticum* were highly efficient against a wide-ranging spectrum of clinic-isolated bacteria compared to antibiotics. Recently, with increased resistance to drugs, pathogen problems and threats, the extract has become an acceptable alternative to antibiotics and the extract plant species are toxic to multidrug-resistant microorganisms.

Anti-adherence and anti-biofilm activity of aqueous extracts of *Syzygium aromaticum*

The Anti-adherence and Anti-biofilm activity of aqueous extracts of *Syzygium aromaticum* against (G-ve) bacteria (Fig. 5), most bacterial isolated of (G-ve) bacteria exhibit moderate adherence and biofilm activity to these extracts and some bacterial isolated of bacteria were exhibit high adherence and biofilm activity to aquatic extracts of *Syzygium aromaticum*.

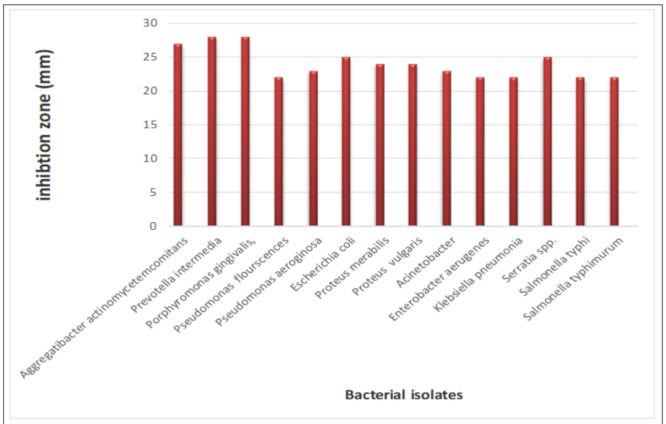

Fig. 1. Antimicrobial effect of *Syzygium aromaticum* against (G-ve) bacteria by agar well method.

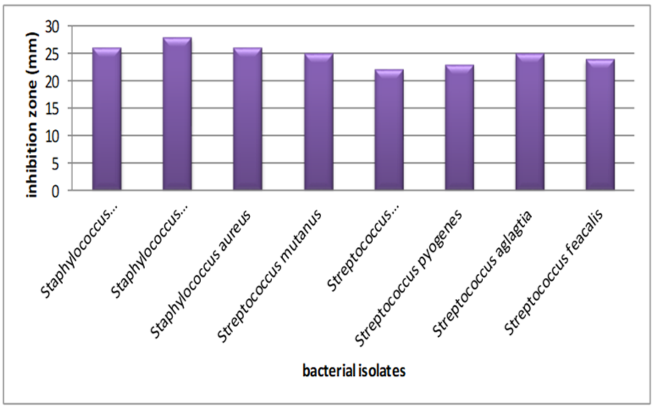

Fig. 2. Antimicrobial effect of *Syzygium aromaticum* against (G-ve) bacteria by agar well method. Some isolated bacteria were sensitive to floxacine.

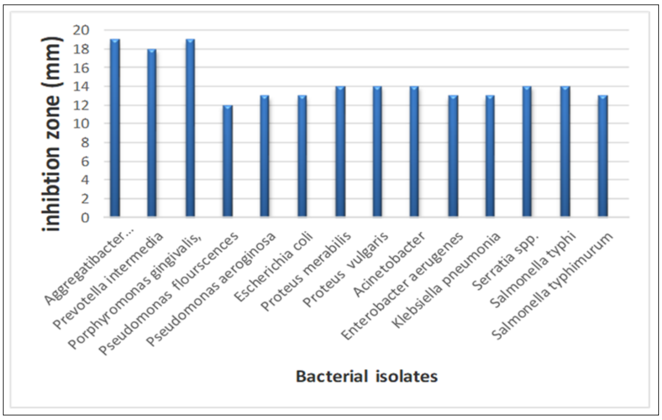

Fig. 3. Antimicrobial effect of floxacine against (G-ve) bacteria by agar well method.

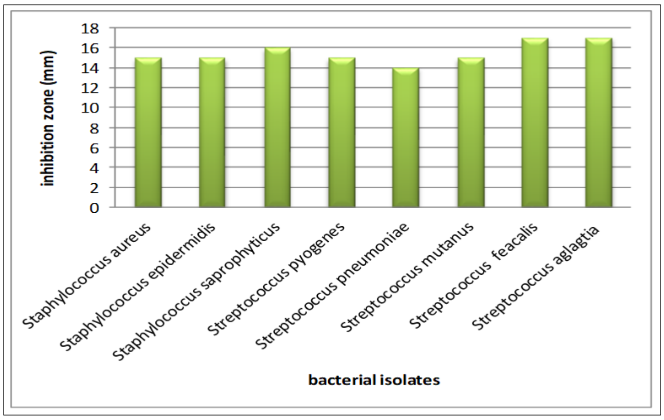

Fig. 4. Antimicrobial effect of floxacin against (G+ve) bacteria by agar well method.

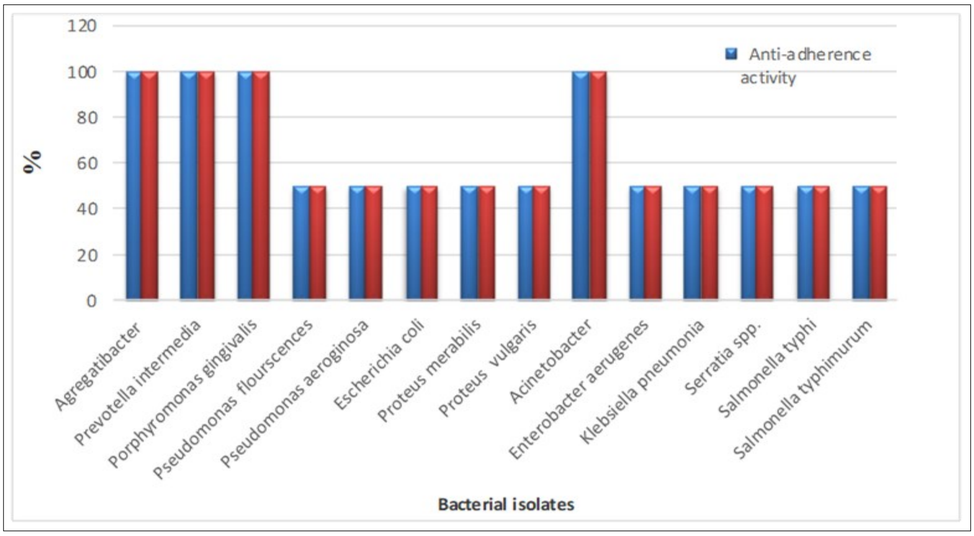

Fig. 5. Anti-adherence & anti-biofilm activity of aqueous extracts of *Syzygium aromaticum* against (G-ve) bacteria.

Based on the experiment results and discussion, it could be assumed that the aquatic extract has potent antimicrobial properties that inhibit biofilm formation, adherence and motility of bacteria to reduce pathogenicity. These extracts can prevent the bacteria that cause urinary tract infections, diarrhoea and dental cavities. They are effective against both G -positive and G-negative bacteria. Regarding this, it is advised to consume the extract since it may prevent bacterial diseases and inhibit the adherence of numerous bacteria. This study could pave the way for more investigation into oral vaccinations that take advantage of bacterial adhesions.

Moreover, the main specific feature of pathogenic bacteria that enables them to survive hard conditions is a biofilm-forming capacity, which may raise the risk of healthcare -associated infections (30). However, biofilm actively participates in bacterial colonization at the infection sites. It is a defence tool that reduces the entrance of antibiotics. In a way, biofilm offers protection to the colonies of isolates. Furthermore, biofilm promotes drug resistance in organisms because of intra-species genetic transfer within biofilms (31,32). The key focus of most biomedical researchers has been to explore the role of antimicrobial-resistant phenotype and biofilm-forming factor and their effect on the health outcomes of infections. Another investigation indicated that microbes at low antibiotic concentrations could produce biofilms in both G-positive and G-negative bacteria, which implied that these microorganisms promoted biofilm formation to overcome external pressure (31).

The result shows that the influences of crude extract on the growth and adherence of pathogenic bacteria of human buccal epithelial cells were studied. Black raisins and their vinegar prevented Pathogenic bacteria from growing or adhering to the buccal epithelial cells (22). Several research studies have indicated that the extract's constituents, such as triterpenes, betulin, oleanolic and betulinic acids, have anti-gum disease and anti-cavity characteristics. Biofilm of pathogenic bacteria has been related to persistent infection due to their high resistance to antimicrobial agents, while the biofilm of beneficial bacteria often boosts the human immune system. Hence, the control forming of biofilm for both pathogenic and beneficial bacteria is essential in bacterium-related diseases.

Conclusion

Syzygium aromaticum extracts were highly efficient against a broad spectrum of clinically isolated (G-ve) and (G+ve) bacteria, indicating they are more effective than commercially available antibiotics. Additionally, they highly inhibited adherence and the forming of biofilm.

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Authors' contributions

All authors contributed equally to writing and approving this manuscript.

Compliance with ethical standards

Conflict of interest : The authors do not have any conflict of interest to declare.

Ethical issues : None

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