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Research Article

Toxicological Effects of Aqueous and Ethanol Extracts of Clove (*Syzygium aromaticum*) on Various Pathogenic Bacteria

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ABSTRACT

Syzygium aromaticum (Clove) is a highly aromatic spice derived from the dried flower buds of the clove tree, which originated in Indonesia. It has been used for centuries in various cuisines and traditional medicine practices worldwide. Medicinal plants, including clove, have been utilized to treat a variety of illnesses caused by bacteria.

This study investigates the antibacterial effects of aqueous and ethanol extracts of *Syzygium aromaticum* on various pathogenic bacteria. The extracts were prepared using standard procedures and their antimicrobial activities were evaluated against selected pathogenic bacteria using the agar well diffusion method.

The results indicated that the ethanol extract of *Syzygium aromaticum* exhibited the highest antimicrobial activity, showing an inhibition zone of 25.5 mm against *Salmonella typhi* at a concentration of 100 mg/mL.

In contrast, the aqueous extract demonstrated lower activity against the same organism. Both extracts showed a Minimum Inhibitory Concentration (MIC) of 6.25 mg/mL against all isolates, except for the aqueous extract against *Escherichia coli* (*E. coli*), which had an MIC of 12.5 mg/mL.

This research suggests that the tested extracts of clove can effectively combat diseases caused by the tested organisms.

Keywords: *Syzygium aromaticum*, Minimum inhibitory concentration, Minimum bactericidal concentration, Toxicologicals

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INTRODUCTION

Clove refers to the dried flower bud of the tropical tree *Syzygium aromaticum*, which is native to Madagascar and Indonesia. Over the centuries, clove has been utilized as a natural remedy for various ailments and diseases, owing to its multitude of medicinal properties (Mittal M, *et al*., 2014).

Notably, clove possesses significant antimicrobial and antioxidant properties, making it effective in combating infections and boosting the body's immune system (Mysoon AlA, *et al*., 2019; Lee KG and Shibamoto T, 2001). In Western medicine, clove has gained widespread use in dentistry as a local antiseptic and painkiller and it is also a common homeopathic remedy for toothaches. Clove, derived from the tree *Eugenia caryophyllata*, is highly valued in the spice trade for its therapeutic properties (Atkinson RG, 2016). The dried aromatic flower buds of *Syzygium aromaticum*, an evergreen tree in the Myrtaceae family, are utilized as a spice and carminative and they help alleviate gastric irritation commonly found in herbal medicines (Singh RI, *et al*., 2015; Agbaje EO, *et al*., 2009).

Various natural products from different origins are used to preserve food against spoilage and pathogenic microorganisms. Clove holds significance in traditional medicinal practices such as Ayurveda, Chinese medicine and Western herbalism for human medicinal purposes (Kumar Y, *et al*., 2014; Fatope MO, 2001). Despite its common use, research on the effects of clove extracts on pathogenic strains of bacteria and the optimal conditions for demonstrating its greatest efficacy is relatively sparse.

Clove is rich in manganese, dietary fiber, vitamin C, vitamin K, fatty acids and calcium, making it an excellent nutritional source. Traditionally, clove oil has been employed for numerous health conditions such as indigestion, general stress, parasitic infestations, coughs, toothaches and blood impurities (Fagere ZO and Magbou AZ, 2016). Other studies have indicated its potential for treating nausea and vomiting, as well as its use in tropical Asia for diverse infections such as cholera, malaria and tuberculosis (Mintah SO, *et al*., 2019).

The projects outlined here seek to address the gap in knowledge regarding clove's antimicrobial properties and provide a clear scientific basis for optimizing its antibiotic effects in medical treatments and food sciences. Cultures of the isolates were prepared and subjected to tests using clove extracts alongside the well-known antibiotic tetracycline as a positive control. By comparing the results achieved by the clove extract with our current understanding of tetracycline's mode of action, we hope to gain insights into how the active compounds in clove work without causing toxicity.

Syzygium aromaticum plays a strategic role across several industries, including pharmaceuticals, cosmetics, food and beverages and other chemical sectors (Cortés RDF, *et al*., 2014). In recent years, there has been a growing focus on the use of clove extracts in treating diseases, with studies indicating their effectiveness against various bacteria. However, the scientific literature supporting these findings is minimal and toxicology studies on the effects of clove extracts on pathogenic bacteria are still underway (Pandey A and Singh P, 2011).

Clove extracts have shown potential against bacteria such as *Staphylococcus*, *E. coli*, *Salmonella typhi* and pneumonia, which are common causative agents of bacterial illnesses (Uddin TM, *et al*., 2021). Antibiotic resistance often arises as a natural adaptation to antibacterial drugs, allowing bacteria to transfer resistance genes either horizontally or vertically to their offspring.

This study aims to investigate the inhibitory effects of clove extracts and determine the MIC and Bactericidal Concentration (BC) of different clove extract concentrations. The focus will be on bacteria that cause intestinal and soft tissue infections in humans, which have been found to easily acquire drug-resistant genes.

Statement of problem

There is a widely recognized increase in the resistance of microorganisms to antimicrobials and a corresponding rise in toxicity rates associated with various pathogens. Many researchers have conducted extensive studies to address and mitigate the toxicological effects on pathogenic bacteria.

Syzygium aromaticum extract is globally acknowledged for its numerous bioactive compounds that inhibit the growth of a wide range of microorganisms. Given this potential, there is a pressing need to conduct research aimed at finding and producing a potent alternative drug from clove extracts of both aqueous and ethanol through *in vitro* susceptibility testing against selected pathogenic bacteria, particularly in cases where orthodox medicine fails.

Aim of the study: The aim of this study is to investigate the toxicological effects of *Syzygium aromaticum* on pathogenic bacteria.

Objectives: The objectives of this study are to confirm the Zone of Inhibition (ZOI) on the pathogenic bacteria, to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extract, and to investigate the potential antimicrobial properties of clove extracts.

Toxicological studies

Toxicological studies are important for determining the safety and toxicity rates of specific drugs. These studies assess the dose rates that may produce serious toxicological manifestations when administered either at once or over multiple doses (Okereke SC, *et al*., 2023).

Medicinal plants generally contain bioactive compounds and clove *Syzygium aromaticum* specifically has a significant secondary bioactive compound known as eugenol, which constitutes 70%-90% of clove extract and is responsible for its characteristic aroma. Although eugenol is considered safe due to its wide range of applications and extensive use, recent concerns about its toxicity have emerged.

The Food and Drug Administration (FDA) has confirmed the safety of both eugenol and clove buds. Research by Prashar A, *et al*., 2006 documented the cytotoxicity activities of Eugenol *in vitro* against human fibroblasts, recognizing it as safe.

Numerous studies have examined the toxicity of *Syzygium aromaticum*, with reviews indicating no recorded toxic rates at doses ranging from 1000 mg/kg to 5000 mg/kg body weight. These studies utilized distilled water and ethanol, with extracts showing no signs of toxicity even at the highest dosages. The World Health Organization (WHO) has established a daily accepted intake for eugenol at a maximum of 2.5 mg/kg body weight; however, most studies have limited the highest dose of clove extract used to 1000 mg/kg (Srivastava AK, *et al*., 2005).

MATERIALS AND METHODS

Source of plant material/collection of herbs

Dried clove *Syzygium aromaticum* was purchased from sabon layi market, Bali, Taraba State. The plant material was authenticated by a botanist from the Department of Biological Science, Federal Polytechnic Bali, Taraba State, Nigeria.

Source of the organisms

The test organisms were collected from the Department of Biological Science, Microbiology Unit, Federal Polytechnic Bali, Taraba State, Nigeria. The test organisms included *Staphylococcus aureus*, *E. coli* and *Salmonella typhi*.

Preparation of clove extract

The cloves were cleaned to remove dirt and washed with distilled water to eliminate dust from the surface. They were then air-dried at room temperature and pounded into a powder using a laboratory mortar and pestle. The powdered cloves were sieved and stored in an airtight container, properly labelled for future use (Mainasara MM, *et al*., 2017).

Preparation of aqueous extract of clove

25 g of the powdered clove was dissolved in 250 mL of distilled water in a conical flask to create a proper mixture. The flask was covered with cotton wool and foil paper and the contents were mixed thoroughly. It was then kept for 48 hours with regular shaking at intervals. The mixture was filtered using Whatman no. 1 filter paper. The filtrate was centrifuged and evaporated to dryness, resulting in a reddish-brown colour and then stored at 4°C in the refrigerator until needed (Sanusi SB, *et al*., 2019).

Preparation of ethanolic extract of clove

Similarly, 25 g of the powdered clove was mixed with 250 mL of ethanol in a conical flask to produce a proper mixture. The flask was covered with foil paper and cotton wool and shaken thoroughly. After being kept for 48 hours, the mixture was filtered using Whatman no. 1 filter paper. The clove mixture was then evaporated to yield a thick dark brown extract, which was stored at 4°C in the refrigerator until required.

Culture media and inoculum

Using a sterile inoculating wire, the cultures of the test organisms were transferred into new media prepared with nutrient agar and incubated for 24 hours at 37°C.

Determination of antibacterial activity

The agar well diffusion method was performed as described by Sanusi SB, *et al*., 2022 for the antimicrobial susceptibility test of *Syzygium aromaticum* extracts. Suspensions of the test organisms were inoculated onto the surface of solidified Mueller-Hinton agar plates using a sterile swab (Ode I, *et al*., 2023)

A steel borer was then used to create six wells, each 4 mm in diameter, on the agar plate. Different concentrations 0.1 mL of the reddish-brown aqueous extract of clove were added to five wells at concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL. Tetracycline was used as a control and added to the sixth well. The prepared Petri dishes were incubated overnight at 37°C.

After incubation, the ZOI was measured using a clear measuring ruler to determine the diameters of inhibition on each plate, thereby evaluating the potential efficacy of the extracts. The same procedure was conducted for the ethanol extracts of clove at the same concentrations: 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL, using tetracycline as a control.

Determination of MIC

The MIC was determined using broth dilution methods with Mueller-Hinton broth for the aqueous extract. Seven test tubes were prepared, each containing 5 mL of broth. To each tube, 1 mL of each extract concentration (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) was added and thoroughly mixed.

0.1 mL of the bacterial culture was introduced into five of the test tubes. The sixth tube received only broth and the test organism as a positive control, while the seventh tube contained only broth as a negative control. All inoculated tubes were then incubated overnight at 37°C to observe bacterial growth.

The MIC of the aqueous extract was defined as the lowest concentration of antimicrobial that inhibits visible growth of test organisms after overnight incubation. This procedure was similarly conducted for the ethanol extracts of clove at concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL.

MBC of Syzygium aromaticum against the isolates

The dilution broth method was used to determine the MBC. A volume of 0.1 mL was removed from the wells of the agar plates where no growth was observed after 48 hours of incubation at 37°C. The MBC was assessed only for the ethanolic extract against *Staphylococcus aureus* and *Salmonella typhi*, both showing an MBC of 6.25 mg/mL. The MBC is defined as the lowest concentration of an antimicrobial that will prevent the growth of an organism after sub culturing onto antibiotic-free media.

RESULTS AND DISCUSSION

The aqueous extract of the test plant at concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL was found to be less active than the ethanol extract (*Tables 1 and 2*). It exhibited weak antibacterial activity against all tested organisms, with the highest activity observed against *Salmonella typhi* of 23 mm at 100 mg/mL, followed by *Staphylococcus aureus* 20 mm and *E. coli* 18 mm at the same concentration. No antibacterial activity was observed at 6.25 mg/mL for any tested organisms.

In contrast, the ethanol extract showed a highest ZOI of 25.5 mm against *Salmonella typhi*, followed by *Staphylococcus aureus* (21 mm) and *E. coli* (20 mm) at 100 mg/mL. Tetracycline, used as a control antibiotic, demonstrated the highest diameter of inhibition on *Staphylococcus aureus* (28 mm) and other organisms (24 mm).

Both aqueous and ethanol extracts of clove exhibited a MIC of 6.25 mg/mL against all tested isolates, except for the aqueous extract against *E. coli*, which showed an MIC of 12.5 mg/mL. The MBC was only observed for the ethanol extract of clove against *Salmonella typhi* and *Staphylococcus aureus*, both at 6.25 mg/mL (*Tables 3 and 4*).

Table 1: Zone of inhibition of aqueous extract of clove (*Sygyzium aromaticum*) in diameter (mm) on the pathogenic bacterial

(ZOI) Varying concentration (mg/ml)						
Organisms	**100 (mm)**	**50 (mm)**	**25 (mm)**	**12.5 (mm)**	**6.25 (mm)**	**TC**
Salmonella typhi	23	17.5	15	10	---	24
Escherichia coli	18	13	9	---	---	24
Staphylococcus aureus	20	15.5	14	8	---	28
Note: ZOI at varying concentration=Number of zone of inhibition; TC: Tetracycline						

Table 2: Zone of inhibition of ethanol extract of clove (*Sygyzium aromaticum*) in diameter (mm) on the pathogenic bacterial

(ZOI) Varying concentration (mg/mL)						
Organisms	**100 (mm)**	**50 (mm)**	**25 (mm)**	**12.5 (mm)**	**6.25 (mm)**	**TC**
Salmonella typhi	25.5	20	16	10	---	24
Escherichia coli	20	17	13	8	---	24
Staphylococcus aureus	21	18	15	9	---	28
Note: ZOI at Varying concentration=Number of zone of inhibition; TC: Tetracycline						

Table 3: Minimum Inhibitory Concentration (MIC) of *Syzygium aromaticum* on pathogenic bacteria

Organisms	EEC (mg/mL)	AEC (mg/mL)
Salmonella typhi	6.25	6.25
Escherichia coli	6.25	12.5
Staphylococcus aureus	6.25	6.25
Note: EEC: Ethanol Extract of Clove; AEC: Aqueous Extract of Clove		

Table 4: Minimum Bactericidal Concentration (MBC) of *Syzygium aromaticum* on pathogenic bacteria

Organisms	EEC (mg/mL)	AEC (mg/mL)
Salmonella typhi	6.25	---
Escherichia coli	---	---
Staphylococcus aureus	6.25	---
Note: EEC: Ethanol Extract of Clove; AEC: Aqueous Extract of Clove		

CONCLUSION

Syzygium aromaticum offer a range of potential health benefits. Consuming cloves may help reduce inflammation in the body, which could be beneficial for conditions such as arthritis, inflammatory bowel diseases and other diseases caused by the test organisms. The MIC values indicated dose-dependent inhibition and bactericidal effects, with lower concentrations required for ethanol extracts compared to aqueous extracts.

RECOMMENDATIONS

This research demonstrates and recommends utilizing clove as a supportive remedy and for the development of new drugs, providing a dose-dependent or daily dosage rate against *Staphylococcal* infections.

REFERENCES

1. Mittal M, Gupta N, Parashar P, Mehra V, Khatri M. Phytochemical evaluation and pharmacological activity of *Syzygium aromaticum*: A comprehensive review. Int J Pharm Pharm Sci. 2014; 6(8): 67-72.
2. Mysoon AlA, Vijayaraghavan P, Ravindran B, Chang SW, Agastian P. Identification of phytochemical components from *Aerva lanata* (Linn) medicinal plants and it's *in vitro* inhibitory activity against drug resistant microbial pathogens and antioxidant properties. Saudi J Biol Sci. 2019; 26(6): 1129-1133.
3. Lee KG, Shibamoto T. Antioxidant property of aroma extract isolated from clove buds *Syzygium aromaticum* (L) Merr. et Perry. Food Chem. 2001; 74(4): 443-448.
4. Atkinson RG. Phenylpropenes: Occurrence, distribution, and biosynthesis in fruit. J Agric Food Chem. 2016; 66(10): 2259-2272.
5. Singh RI, Lawrence RE, Lawrence K, Agarwal B, Gupta RK, Dar S. Antioxidant and antibacterial activity of *Syzygium aromaticum*, *Zingiber officinale* and *Cinnamomum zeylanicum* essential oils. Chem Sci Trans. 2015; 4(1): 239-245.
6. Agbaje EO, Adeneye AA, Daramola AO. Biochemical and toxicological studies of aqueous extract of *Syzigium aromaticum* (L) Merr. & Perry (Myrtaceae) in rodents. Afr J Tradit Complement Altern Med. 2009; 6(3).
7. Kumar Y, Agarwal S, Srivastava A, Kumar S, Agarwal G, Khan MZ. Antibacterial activity of Clove (*Syzygium aromaticum*) and Garlic (Allium sativum) on different pathogenic bacteria. Int J Pure App Biosci. 2014; 2(3): 305-311.
8. Fatope MO. Looking back and looking forward. Nat Prod Sci. 2001.
9. Fagere ZO, Magbou AZ. Antibacterial activity of clove oil against some microorganisms at Khartoum state, Sudan. Adv Med Plant Res. 2016; 4(4): 122-128.
10. Mintah SO, Asafo AT, Archer MA, Junior PA, Boamah D, Kumadoh D, *et al*. Medicinal plants for treatment of prevalent diseases. Pharmacogn Med Plants. 2019. 1-9.
11. Cortés RDF, de Souza CR, Oliveira WP. Clove (*Syzygium aromaticum*): A precious spice. Asian Pac J Trop Biomed. 2014; 4(2): 90-96.
12. Pandey A, Singh P. Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. Asian J Plant Sci Res. 2011; 1(2): 69-80.
13. Uddin TM, Chakraborty AJ, Khusro A, Zidan BR, Mitra S, Emran TB, *et al*. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. J Infect Public Health. 2021; 14(12): 1750-1766.
14. Okereke SC, Edom VC, Nwaogwugwu CJ, Aaron CF, Oko IP, Obinna IB, *et al*. Toxicological assessment of *Irvingia gabonensis* leaf extracts in albino rats: A comparative study between aqueous and ethanol extraction methods. Adv Biol Chem. 2023; 13(4): 143-170.
15. Prashar A, Locke IC, Evans CS. Cytotoxicity of clove (*Syzygium aromaticum*) oil and its major components to human skin cells. Cell Prolif. 2006; 39(4): 241-248.
16. Srivastava AK, Srivastava SK, Syamsundar KV. Bud and leaf essential oil composition of *Syzygium aromaticum* from India and Madagascar. Flavour Fragr J. 2005; 20(1): 51-53.
17. Mainasara MM, Sanusi SB, Maishanu HM, Ismail T. Antibacterial activity and nutritional content of fresh and dried date fruits (*Phoenix dactylifera*) L. Int J Sci Healthcare Res. 2017; 2(1): 15-20.
18. Sanusi SB, Audu Y, Hamza I, Usman A, Makama P. Phytochemical analysis and antibacterial activities of ginger *Zingiber officinale* collected from different parts of Kaduna state against selected bacteria isolated from wound. Sci World J. 2019; 14(4): 62-65.
19. Sanusi SB, Lawal SM, Usman A, Musa FM, Ardo B, Way TB. Phytochemical analysis and antibacterial activity of stem bark extracts of *Detarium microcarpum* against bacteria causing gastrointestinal tract infections in humans. Dutse J Pure Appl Sci. 2022; 8(1): 82-89.
20. Ode I, Wahjuningrum D, Yuhana M, Setiawati M. The antibacterial activity of clove *Syzygium aromaticum* extract and its effects on the survival rate of hybrid grouper *Epinephelus fuscoguttatus*♀× *E. lanceolatus*♂ infected with *Vibrio alginolyticus*. J Akuakultur Indones. 2023; 22(1): 1-1.