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Antibacterial and Antifungal Activity of Clove Extract (*Syzygium aromaticum*): Review

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A B S T R A C T

Cloves is one of the native Indonesian plants and is used in many aspects of life. Cloves are used in multiple industries and as detergents, soaps, perfumes, food seasonings, aromatherapy, etc. Cloves are mainly used for ingredients in kretek cigarettes in Indonesia. Various studies of cloves reported they have good pharmacological and therapeutic effects. The main compounds of clove extract are eugenol and β-caryophyllene, which are powerful antibacterial and antifungal agents. The clove ethanolic extract showed the activity to inhibit Gram-positive and negative bacteria such as B. cereus, S. aureus, E. coli, P. aeruginosa, S. pneumonia, S. aureus,S. epidermidis, A. hydrophila, K. pneumoniae, P. gingivalis, and P. mirabilis . Clove essential oil has shown the ability to inhibit the growth of V. inaequalis, C. Albicans, C. glabrata, and C. tropicalis. Cloves extract can be used as an essential ingredient in various medicines. However, it requires further research and trials.

1. Introduction

Antibiotics and antifungal drugs are the most important treatments to fight pathogenic microbes. The incidence of fungal and bacterial infections has increased leading to the increasing usage of antibiotics and antifungal medicines. Free use of antibiotics and antifungals without adhering to the rules of rational use of medicine causes resistance in bacteria and fungi. It is essential to find a new alternative drug with lesser resistance. Plants are natural sources that play an important role in the prevention and treatment of human diseases. Herbal medicine is one of the healthcare systems in developing countries, such as Indonesia. Herbs are widely exploited as traditional medicine in Indonesia. Their curative potential is also well-documented. Herbal medicine is not only used as a drug, but also as a natural antiseptic.[1-4]

Syzygium aromaticum is one of the alternative candidates for herbal medicine belonging to the Myrtaceae family. This plant comes from Maluku spreading throughout Indonesia. The *Syzygium aromaticum* plant grows up to 8-12 meters tall. The leaves and the flowers grow in groups. Cloves are aromatic flower buds and the generally known part of the Syzygium aromaticum plant that is used. Cloves are dried before use, so they are durable and easy to sell. Cloves are usually used as a spice and as the basic ingredient of kretek cigarettes. Cloves are also well-known for their pharmacological benefits. One of the examples of its pharmacological benefits is that

cloves are often used to treat toothache pain. Clove extracts are also used as antiseptics to kill bacteria. Therefore, clove extracts can be used as an antibacterial agent. The objective of this review was to find out the potency of bioactive clove extracts as antibacterial and antifungal agents.[5-9]

2. Methods

PubMed and Google Scholar websites were used to find the articles needed. The article search was conducted until May 25, 2020. The keywords were "Syzygium aromaticum" and "antibacterial" or "Syzygium aromaticum" and "antifungal". The articles were limited to the last ten years. We obtained 198 articles from PubMed and 26 from Google Scholar. Inclusion criteria for this study were the relevant articles related to Syzygium aromaticum and the study of antibacterial and antifungal agents. There were 179 articles excluded because of duplication and/or out of topic. There were 47 articles that met the criteria. A summary of the article's selection is shown in Figure 1.

3. Discussion

The bioactive compound of cloves extract

There are many types of clove extracts such as ethanol extracts, essential oil, etc. The bioactive compound of these extracts varies. Singletary et al. compared various compounds from various types of clove extracts, such as essential oils, ethanol extracts, hexane extracts, and water extracts. The bioactive compound of each clove extract is different (Table 1). The extraction methods and the solvent used determine the bioactive compound obtained. Eugenol and β-caryophyllene are the main bioactive compounds found in the respective extracts.[5]

Clove oil is usually used in aromatherapy and some medicine. It is usually applied to relieve toothache. The phytochemical of the essential oil plays the important role in the activity. Raatjkoswa et al. and Dianez et al. reported that the main compounds of the cloves' essential oil are eugenol (around 85%) and β-caryophyllene (around 9%).[7,8]

Eugenol [2-methoxy-4-(2-propenyl)phenol] is an active compound found naturally in various herbal plants, especially cloves (Figure 2). Eugenol is often used as a pain reliever because of its anesthetic effect. It is typically applied to a cavity in a decayed tooth or tooth socket. Eugenol is commonly used as aromatherapy and flavoring food and as an ingredient in perfumes, soaps, detergents, food, and beverage.[9-13]

The chemical structure of eugenol is related to phenol. It is an antioxidative agent and can be used to prevent diseases caused by free radical activity, such as cancer, cardiovascular disease, neurodegenerative diseases, and others. Fujisawa et al. reported that eugenol acts as an anti-inflammatory and anti-proliferative agent. Eugenol exhibits strong antibacterial and antifungal activity.[14]

β-Caryophyllene is a natural bicyclic sesquiterpene. It is one of the main compounds found in cloves (around 9%) besides eugenol (Figure 2). This compound exhibits some potential activities such as anti-inflammatory, neuroprotective, antidepressant, anti-allergic, antioxidant, and anti-tumor. The pharmacological and therapeutic effects indicate that β-Caryophyllene is beneficial to human health.[15-18]

β-Caryophyllene has strong antioxidant and anti-inflammatory activity. β-Caryophyllene improves systemic inflammation and increases the oxidative status of arthritis mice. β-Caryophyllene can be used as an anticancer agent and analgesic compound. The anticancer mechanism is not well-explained, so it needs further research.[19,20]

Antibacterial activity

Pathogen bacteria cause infections and impair human health. Bacterial transmission can go through various ways such as air, food, and water media. Some of the examples of bacteria that can cause a foodborne disease are *S. aureus* and *B. cereus*.[21]

Clove essential oil has a strong antibacterial activity to prevent bacterial growth. It showed its ability against *A. hydrophilia* ATTC 7966, *C. Albicans* ATTC 10231, and *P. mirabilis* ATTC 10005 by the agar well diffusion method. The clove essential oil also

exhibits the ability to inhibit the growth of *S. aureus* ATTC 6538, *S. epidermidis* ATTC 14990, *E. coli* ATTC 8739, *P. aeruginosa* ATTC 27853, and K. pneumonia ATTC 13883. Whereas the clove essential oil against *E. faecalis* ATTC 2912 and *S. pyogenes* ATTC 1915 had no antibacterial activity.[21]

Food is one of the bacterial transmission media that causes food poisoning, which can cause severe illness and death in humans. Chemical preservation is usually used to prevent spoilage. Repeated application of the chemical preservative leads to bacterial resistance. The development of natural resources as antimicrobial agents has been done to avoid bacterial resistance. Clove exhibits the ability to prevent bacterial growth. Ethanolic extract from cloves was effective in inhibiting the growth of several bacteria. The minimum inhibitory concentration is 10 mg/ml and the inhibition zone of B. cereus, S. aureus, P. aeruginosa, and E. coli are 14.6±0.37 mm, 15.8±0.41 mm, 13.4±0.11 mm and 11.9±0.34 mm, respectively. These results can be compared to the positive control Gentamycin (5 µg. The zone inhibition of Gentamycin in *B. cereus, S. aureus, E. coli,* and P. aeruginosa are 16.8±0.37 mm, 20.5±0.24 mm, 15.6±0.53 mm, and 13.1±0.35 mm, respectively. Ethanolic clove extract showed no inhibition activity against *Salmonella typhi.*[22]

Streptococcus pneumonia bacteria causes pneumonia and otitis media in humans. Eugenol shows strong inhibitory activity against S. pneumonia with MIC and MBC rates of 0.06% and 0.12%. Eugenol 0.6% exhibits the ability to prevent the growth of 99% of bacteria in 30 minutes and 99.99% of bacteria were killed by eugenol 0.12%. Although not as good as the eugenol solution, clove extract can also inhibit these bacteria's growth. Based on this research, eugenol solution has a more vital inhibiting ability than clove extract. The lower inhibitory ability of the clove extract than the eugenol solution is probably due to the clove extract's impurity.[23]

Clove essential oil showed strong antibacterial activity against P. gingivalis which causes periodontal disease. The ability to inhibit bacterial growth can be seen in the MIC 6.25 µg/mL and MBC 25 µg/mL. This result can be compared to the positive control Tinidazole (MIC 7.8 µM and MBC 7.8 µM). Eugenol plays an important role in inhibiting bacteria growth. Eugenol increases cell membrane permeability. It improves the transport of ATP and K+ to the outer membranes and leads to the destruction of cell membranes.[24]

There are some inhibition mechanisms of eugenol and clove essential oil to prevent the growth of various bacteria, such as inhibiting bacterial migration, bacterial adhesion, bacterial metabolism, and bacterial invasion. The mechanism has been examined by observing the antibacterial activity of the clove extract against E. coli. The strongest activity was detected during the first 10 minutes after the eugenol was given. Eugenol suppressed the expression of genes related to migration or movement of the bacteria, the adhesion and the colonization, such as fliC, fimA, lpfA, and hcpA. The fliC is a gene that encodes flagellin A which is important for bacteria movement. FimA gene encodes fimA protein composed of fimA fimbriae that are important for adhesion and colonization. The lpfA and hcpA genes are also associated with cell adhesion. Eugenol suppresses the expression of the virulence genes Shiga toxin 1 (stx1) and Shiga toxin 2 (stx2), which encode the virulence protein Verotoxins 1 (VT1) and Verotoxins 2 (VT2). Eugenol also downregulates the eae and Ler genes that are functional for bacterial adhesion. The summary of antibacterial activity mechanisms can be seen in Table 3.[25]

Streptococcus sp. initiates dental plaque formation. Streptococcus mutans is the main cause of dental plaque and caries. Some researchers reported that dental caries is a classic biofilm disease that occurs due to oral environment changes. β-Caryophyllene showed the ability to inhibit Streptococcus mutants' growth. The concentrations used are above 0.078%. β-Caryophyllene showed the activity to inhibit the expression of glucosyltransferase and suppressed the cariogenic biofilm. β-Caryophyllene can be a candidate for preventing dental caries.[15] The antibacterial

activity of clove extract and the essential oil can be seen in Tables 4 and 5.

Antifungal activity

Clove essential oil has shown the ability to inhibit the growth of V. inaequalis, C. Albicans, C. glabrata, and C. tropicalis. *Venturia inaequalis* is a fungus that causes apple scab disease in apple plants. Muchembled et al. conducted a study on the antifungal properties of various essential oils. The results showed that clove essential oil is the most effective compound in inhibiting V. inaequalis, and the dominant bioactive compound of this essential oil is eugenol.[26]

Cloves of essential oil obstruct the biofilm formation. The inhibition effect of the clove essential oil on C. Albicans was 47.61%. The result can be compared to the fluconazole, with an inhibition activity of 48.16%. Electron microscope analysis showed the disorganization of the biofilm formed and the inhibition of filament formation. This filament causes fungal cells to escape easily.[27]

Antifungal activity of the clove extract was observed against C. Albicans, C. glabrata, and C. tropicalis. There were four solvents used, including methanol, ethyl acetate, n-hexane, and diethyl ether. All extracts exhibited antifungal activity. Ethyl acetate showed the strongest antifungal activity compared to the other extracts. Eugenol and eugenol acetate were the dominant bioactive agents against C. albicans.28

Eugenol inhibits the biosynthesis of aflatoxin B1 (AF B1) by 95.4% by downregulating the transcription of aflatoxin biosynthesis genes, such as aflR, aflT, aflD, aflM, and aflP. Aflatoxin is a carcinogenic compound produced by Aspergillus flavus. Eugenol also inhibits Aspergillus parasiticus at a concentration of 150 ppm and completely inhibits the growth of A. parasiticus after 20 days of incubation.[25]

Eugenol has a strong activity to inhibit C. Albicans keratitis in rabbits. Keratitis is an inflammation of the cornea caused by infection, injury, or disease. The MIC of eugenol against C. Albicans was 2 mg/mL. It can be compared to fluconazole (MIC >0.4 mg/mL). Seventy-five percent of rabbits treated with eugenol successfully recovered from keratitis.[29]

Essential oil of cloves showed the inhibition activity of *Saccharomyces cerevisiae* growth. The MIC of clove essential oil and eugenol were 0.32 and 0.64 ml/mL, while the MBC of these two ingredients were 0.64 and 1.25 ml/mL. The antifungal activity that occurs is based on the lesion of the cell membrane and the reduction in ergosterol quantity, which is a specific component of the fungal cell membrane. The main targets of eugenol against S. cerevisiae are the biosynthesis and transport of amino acids. Eugenol also showed the inhibitory effect of Tat1p and Gap1p, which are proteins that play a role in amino acid transport.[25]

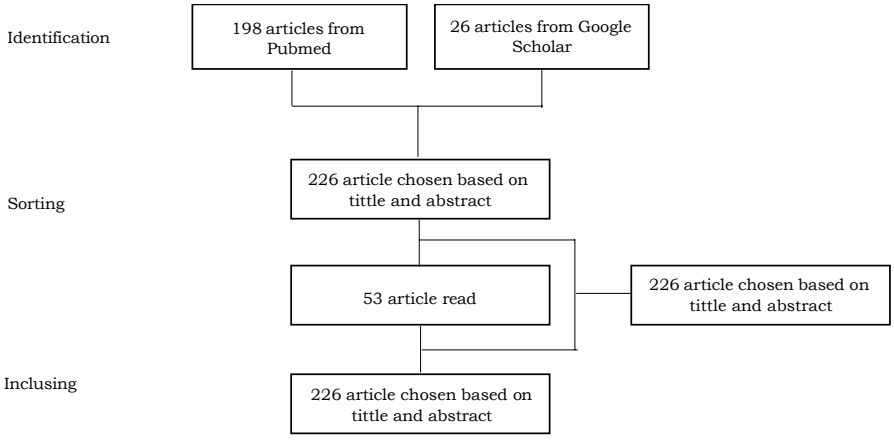

Figure 1. Articles' selection process.

Table 1. The bioactive compound of various clove extracts[5]

Clove Extracts	Compound Content
Essential Oil	- Eugenol - β-caryophyllene - α-humulene - Eugenyl Acetate
Ethanol Extract	- Eugenol - β-caryophyllene - Eugenol Acetate - Flavonoids - Tannins - Alkaloids
Hexan Extract	- Eugenol - Eugenol Acetate - β-caryophyllene - Flavonoids
Water Extract	- Eugenol - Trans-caryophyllene - Anthraquinones - Saponins - Flavonoids - Tannins - Eugenol derivates

Table 2. The bioactive compound of cloves essential oil[7,8]

	Ratjkowska *et al.* (2019)	**Dianez *et al.* (2018)**
Clove Essential Oil Compounds	Eugenol (85.2%)	Eugenol (86.38%)
	β-caryophyllene (9.9%)	β-caryophyllene (9.05%)
	α-humulene (1.9%)	α-humulene (2.61%)
	Caryophyllene oxide (0.4%)	Caryophyllene oxide (1.67%)
	δ-Cadiene (0.4%)	δ-Cadiene (0.29%)

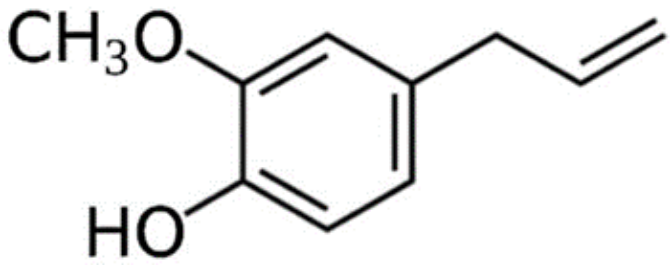

Figure 2. Eugenol structure[5]

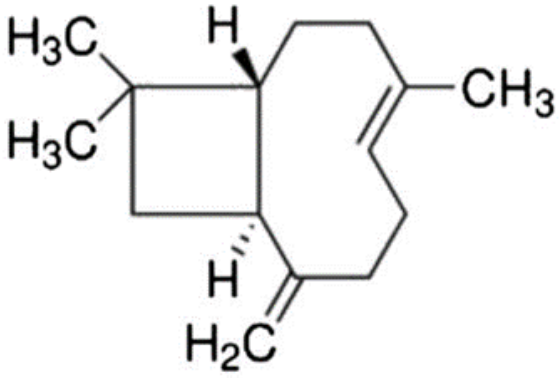

Figure 2. β-Caryophyllene Structure[5]

Table 3. Mechanisms of antibacterial activity of eugenol and clove essential oil[25]

Extract	Bacteria	Antibacterial Activity
	Escherichia coli	- Inhibits bacterial migration - Inhibits bacterial adhesion - Inhibits the expression of virulence factors - Inhibits fimbriae formation
	Salmonella enteritidis	- Inhibits bacterial migration - Inhibits adhesion and bacterial invasion - Inhibits bacterial metabolism - Inhibits the integrity of cell walls and cell membranes
Eugenol		
	Staphylococcus aureus	- Inhibits biofilm formation - Inhibits the expression of virulence factors
	Streptococcus mutans	- Inhibits bacterial adhesion - Inhibits biofilm formation
	Listeria monocytogenes	- Inhibits bacterial adhesion - Inhibits biofilm formation
Essential Oil	*Pseudomonas aeruginosa*	- Inhibits virulence factor expression, migration, and biofilm formation - Inhibits quorum sensing

Table 4. Antibacterial activity of ethanol extract of clove on various bacteria

Gram + Bacteria	Antibacterial Activity	Reference
Bacillus cereus (+)	Yes	22
Staphylococcus aureus (+)	Yes	22
Gram - Bacteria	**Antibacterial Activity**	**Reference**
Escherichia coli (-)	Yes	22
Salmonella typhi (-)	No	22
Pseudomonas aeruginosa (-)	Yes	22

Table 5. Antibacterial activity of clove essential oil on various bacteria

Gram + bacteria	**Antibacterial activity**	**Reference**
Streptococcus pneumoniae (+)	Yes	21
Staphylococcus aureus (+)	Yes	23
Staphylococcus epidermidis (+)	Yes	23
Enterococcus faecalis (+)	No	23
Streptococcus pyogenes (+)	No	23
Gram - bacteria	**Antibacterial activity**	**Reference**
Aeromonas hydrophila (-)	Yes	23
Escherichia coli (-)	Yes	23
Pseudomonas aeruginosa (-)	Yes	23
Klebsiella pneumoniae (-)	Yes	23
Porphyromonas gingivalis (-)	Yes	24
Proteus mirabilis (-)	Yes	23

Table 6. Summary of antifungal activity of cloves essential oil on various types of fungi

Fungi	**Antifungal Activity**	**Reference**
Venturia inaequalis	Yes	26
Candida albicans	Yes	27
Aspergillus flavus	No	25
Aspergillus parasiticus	Yes	25
Saccharomyces cerevisiae	Yes	25

5. Conclusions

Cloves essential oil contains Eugenol and β-Caryophyllene as the main compounds. These compounds are related to antibacterial and antifungal activity. The ethanolic extract has an activity to inhibit the growth of gram-positive and negative bacteria. The essential oil inhibits the growth of bacteria and fungi.

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