Investigation And Development Of Anti Polymicrobial Biofilm From Several Essential Oils: A Review

Hasyrul Hamzah 1,*, Tsaniya Ukhti Nabilah 1, Indra Yudhawan 2, Khalish Arsy Al Khairy Siregar 1, Suci Fitriani Sammulia 3, Fitriani 4

1 Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda, 75124, East Borneo, Indonesia
2 Department of Pharmacy, Faculty of Health Sciences, Universitas Jenderal Soedirman, Purwokerto, 53123, Indonesia
3 Program Study of Pharmacy, Health Institute of Mitra Bunda, Batam, 29444, Riau Island, Indonesia
4 Samarinda Industrial Research and Standardization Center, East Borneo, Indonesia

* Correspondence: hh241@umkt.ac.id

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Abstract: A biofilm is a group of microbes covered with extracellular polymeric substances (EPS) matrix and attached irreversibly to any surface. Biofilm can protect microbes, so microbe could resist the antimicrobial agent and spare from the host immune system. The development of biofilm could be spurred with the occurrence of serum and saliva. Biofilm developed along with increasing clinical infection, so that biofilm also acts as virulence and resistance factors. Furthermore, there are changes in phenotype such as growth rate and gene transcription change in free cell and planktonic cells. Biofilm is involved in many contagious diseases and resistance to various drugs, so it is essential to search and discover a new antibiofilm agent that could inhibit and eradicate biofilm formation. Some discovery a few years ago found that compound from the natural product has chemopreventive and antimicrobial activity in the modulation of biofilm formation. This review summarizes several current research studies related to infection of polymicrobial biofilm and searches for natural polymicrobial antibiofilm with a precise mechanism. The current antibiofilm agents listed here are promising candidates and could give a new approach to managing the infectious disease with polymicrobial biofilm.

Keywords: Biofilm; Antipolymicrobial; Essential Oil; Masoyi; Cinnamon; Thymol; Eugenol

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1. Introduction

Polymicrobial biofilm community is defined as a group of various organisms (bacteria, virus, and fungi) present in the surface and coated within and derived from a hydrated host matrix, often composed of polysaccharides [1–3]. Polymicrobial biofilm frequently consists of a bunch of bacteria, fungi, and viruses. Synergistic interaction in the polymicrobial biofilm impacts the bacterial distribution and overall biomass. Interaction in polymicrobial biofilm influences biofilm physiological function with the increasing resistance, virulence, or pollutant degradation, which could highly affect human health and activity [4–7]. The presence of polymicrobial infections has important implications in disease management because it can modify the clinical course of the disease [8]. A disease related to polymicrobial infections from several infective agents is referred to as complex, complicated, mixed, multiple, synergistic, and concurrent clinical or pathological manifestation. This impacts the choice of antimicrobial therapy and the response to be anticipated, especially pathogens that are usually resistant to an antimicrobial agent [9–11]. Polymicrobial biofilms usually consist of eukaryotic and
prokaryotic pathogenic bacteria [12–14]. Therefore, these biofilms are usually very difficult to diagnose, and their treatment requires complex multi-drug treatment strategies [15–17]. Thus, long-term studies are needed to understand pathogenic biofilm infection because the microbial species in polymicrobial biofilms vary widely [18–20]. This review briefly summarizes the polymicrobial biofilm formation and research in antibiofilm agents derived from the natural product. Furthermore, the mechanism of the antibiofilm agent is also discussed here.

2. Secondary Metabolite

A secondary metabolite is a metabolite compound that is not essential to the growth of an organism, usually synthesized in small amounts even though it has a vital role [21–24]. Secondary metabolite did not directly affect the growth and reproduction of an organism, but it usually has other functions such as propagation, defense, protection and survival [25]. Secondary metabolites are discovered in various forms, depending on one species and another. These are secondary metabolites: alkaloid, phenol, saponin, terpenoid, steroid, tannin, glycoside, flavonoid, etc. [24,26]. Chemical content from the same species could be different because of differences in the place of growth. This phenomenon is called chemoderm. The secondary metabolite, which comes from the natural product, resulted from various factors, both inherent (genetic) or external (environment) factors [5,27]. Several factors could affect secondary metabolites in plants. The amount of secondary metabolite content in the natural product cannot be guaranteed to be constant between one species to another because of that factors.

Several factors are also causing the difference in chemical content. The external factors include soil nutrients, water, temperature, altitude, plants that grow around (allelopathy), and sunlight. The internal factor that comes from the plant environment itself includes pest and infection [24,28]. Plant synthesizes various secondary metabolites with a complex chemical composition [29]. It is produced to respond to various factors such as biotic stress and to support crucial physiological functions such as attracting pollinators, building symbiosis, and providing a structural component for blood vessel tissue cell wall lignification [30,31]. The amount of secondary metabolite in nature is enormous [32]. There are more than 200,000 structures of natural product or secondary metabolites.

To make it easier for learning, classification should be made to simplify the enormous amount and types of secondary metabolites, chemical structure, biosynthesis pathway, and many other classifications. There are many secondary metabolites produced by plants used by the pharmaceutical industry because these bioactive compounds trigger pharmacological or toxicological effects in humans and animals. Secondary metabolites were also used in cosmetics nutrition to manufacture drugs, dyes, fragrances, flavorings, and food supplements. Therefore, the interest in secondary plant metabolites for scientific and industrial purposes is enormous [33–37].

3. Biofilm Formation Process

The biofilm formation process consists of five stages (Figure 1). In the first stage, bacterial cells stick to the surface of the substrate due to the influence of Van der Waals forces. At this stage, the cell adhesion process is still temporary. In the second stage, bacterial cells have stuck permanently due to the formation of the exopolymer material, which could act as a more vital adhesive compound [38]. The formation of microcolonies marks the third stage [39–
Also, biofilms begin to form. In the fourth stage, more and more biofilms are formed, and three-dimensional structures containing shrouded cells were developed within several groups connected [42]. In the last stage, the development of the biofilm structure results in the cell dispersion stage so that the cells are released from the biofilm, attach to new substrates and form new biofilms [43–46].

Hamzah et al. have described the biofilm formation process as stated in their paper [47]. In this paper, in line with that described by hasyrul, we could describe the biofilm formation process in figure 1 as follows: (1) Bacterial cells stick to the surface (temporary); (2) The formation of an exopolymer material; (3) Microcolonies were formed, and biofilms began to form; (4) More and more biofilms are formed; (5) The occurrence of cell disperse so that these cells move and form new biofilms.

The formation of biofilms from multispecies microbes, both bacteria (Gram-positive and Gram-negative) and fungi, especially from the genus Candida albicans, is responsible for the incidence of disease in humans [48–50]. Some literature explains that bacteria can synergistically form biofilms with other bacterial species, causing biofilm structure become physically and physiologically thicker and stronger [51–55].

4. Anti-Polymicrobial Biofilm from Essential Oil (Natural Product)

4.1. Eugenol.

Eugenol is contained in cloves. Eugenol is a liquid that has pale yellow color or is colorless. When exposed to light, its color will change dark brown with a specific smell [4,56,57]. Eugenol is also found in cinnamon (Cinnamomum zeylanicum). Eugenol was known to have antibacterial effects in vitro [58,59]. Also, Eugenol was known to have an antibiofilm effect [60]. This is due to the presence of monoterpenes hydrocarbons that can deactivate enzymes. Eugenol also could react with cell membrane activity, disrupt the genetic material functionality, disrupt the formation of energy production, and disrupt the synthesis of structural components [61,62].
Hamzah has researched *Eugenol* as a polymicrobial antibiofilm state that *Eugenol* was able to inhibit biofilm formation at the intermediate and maturation phases and has the activity to eradicate biofilms formed by a group of microbes (*S. aureus, E. coli, P. aeruginos*, and *C. Albicans*) [4,63,64]. Also stated that *Eugenol* showed concentration-dependent antibiofilm activity in single- and mixed-biofilms formed by drug-resistant strains (*C. albicans and S. mutans*) through multiple modes [65,66].

**4.2. Thymol.**

*Thymol* is a compound isolated from the plant of the Thyme genus, namely *Thymus Vulgaris, Thymus zygis, Thymus citriodorus* [67]. Oregano oil and its main phenolic components Carvacrol [2-methyl-5- (1-methyl ethyl) phenol] and *thymol* (2- isopropyl 5-methyl phenol), are well known for their broad-spectrum antimicrobial activity and have been used as the subject of several in vitro studies [68,69]. Research on *thymol* as a polymicrobial antibiofilm has been conducted by Hamzah states that *thymol* was able to inhibit biofilm formation in the mid-maturation and maturation phases, and has the activity to eradicate mixed biofilms formed by *S. aureus, E. coli, P. aeruginos*, and *C. albicans* [4,70]. Investigating the effect of *thymol* (and *Carvacrol*) on biofilm formation against different carbapenemase-producing Gram-negative bacilli [71]. *Thymol* has an antibiofilm effect from 125-500 μg/mL. Some proposed mechanism of *carvacrol* (and *thymol*) is it can disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides (LPS) and increasing the permeability of the cytoplasmic membrane. Test the Anti-Quorum (AQ) Sensing activity of *thymol*-carvacrol chemotype oil from Lippia organoids with violacein production screening method using CV026 strain [72–74].

![Molecular structure of thymol.](image)

The result showed that *thymol-carvacrol* chemotype oil has a significant reduction in pigment (violacein) production. However, the reduction of pigment was not because of the activity in bacterial viability but probably because of the biological effect caused by the essential oils, stated that *thymol* dose-dependently acts as an antibiofilm agent against UPEC (*Uropathogenic Escherichia coli*) [75–77] *Thymol* at 0,01% v/v could inhibit UPEC biofilm formation on the bottom of the glass dish, confirmed with microscopic observation and COMSTAT analysis. More specifically, *thymol* at that concentration could inhibit biomass, mean thickness, and substratum coverage of UPEC. Also, *thymol* could prevent fimbriae production and swarming motility. Fimbriae (including curli and pili) are critical of biofilm formation by UPEC. Hemagglutination of human red blood is a critical virulence factor in UPEC infections, and adhesive pili have been implicated in the agglutination of erythrocytes.
Thymol could also diminish the hemagglutination ability and reduce UPEC survival in human blood.

4.3. Masoyi (M. aromatica Becc.)

Masoyi (M. aromatica Becc.) is a plant from Indonesia, generally found in the Maluku and Papua regions. Masoyi (Cryptocarya massoy (Oken) Kosterm) belong to Lauraceae family and is often found in the archipelago [79]. It has various names such as C. massoia (Becc.) Kosterm; M. aromatica Becc.; Cinnamomum xanthoneuron Blumedan. On the island of Java, these plants are spread at an altitude of 1,000-1,500 masl. In Maluku, it can be found in South Seram (Seram Selatan), Bacan and especially on the islands of Aru and Kai.

Masoyi bark has a brown color, a very sharp distinctive smell, and a bitter taste [80,81]. The local names of masoyi plants are: masoiyi, masoi wood, mesayi, mangsoi, masuwi, maswi [82]. Masoyi is commonly used to treat asthma, coughs, intestinal worms, stomach pain, swelling, fever, back pain, and gout [82]. Some species belong to the genus of M. aromatica Becc. has been used widely as a traditional medicine in several ethnobotanical and phytochemical practices. Pharmacological studies show that the chemical content consists mostly of pyrones and styrylpyrones, which could exhibit anticancer, larvicidal, and anti-fertility activities [83][84]. Masoyi oil is an essential oil that contains lactone compounds consisting of C-10 and C-12 lactones.

Masoyi oil also contains eugenol, a tanning agent, and resin. Massoialactone is isolated from the skin of Cryptocarya massoia, which is another name for M. aromatica [85]. Massoialactones have 10, 12, and 14 carbon chain compounds called C-10, C-12, and C-14 massoialactones. In nature, this compound is a rare essential oil component that Abe first characterized in 1937 [83,86], and the composition of massoialactone compounds in masoyi peels from the Epa region, Papua New Guinea was reported. The composition are 65% C-10 massoialactone (5,6-dihydro-6-pentyl-2H-pyran-2-one) and C-12 (5', 6-dihydro-6-heptyl-2H-pyran-2-one) as much as 17%, which was detected by Gas Chromatography-Mass Spectrometry (GC-MS). There were also 1.4% C-14 massoialactone (5,6- dihydro-6-nonyl-2H-pyran-2-one) and 2.5% C-10 derivatives (dek-dexalactone) in the hardwood.

C-10 massoialactone is the main compound, found in equal amounts in the bark and stems, and a small amount in fruit oil. Masoyi stem also contains C-14 massoialactone, which
is not found in the bark or fruit of the masoyi. Masoyi oil can irritate the skin or cause sensitization, irritate mucous membranes, sore or damaged skin, and should not be used in a person who is hypersensitive to masoyi oil, children under age 2 years, and the application must be on mucous membranes. The Maximum level for dermal application is 0.01% v/v [81]. Massoialactone could irritate the skin, but it shows good antimicrobial activity against *S. aureus*, *B. subtilis*, and *E. coli* [85].

Several studies reports that essential oils are obtained from the bark of the masoyi stem, stem, and fruit. This plant has a long history as traditional medicine [83,87,88]. The essential oil derived from the *M. aromatica* Becc plant can inhibit 50% biofilm formation from *S. aureus* and *P. aeruginosa* at a concentration of 0.03% v/v. Higher concentrations (0.12% v/v) can disrupt 50% of biofilms that have been formed [86]. Research conducted by Hertiani showed that C-10 massoialactone, oil, and masoyi extract were able to increase macrophage phagocytosis activity and showed activity as an antibiofilm against *C. albicans* with IC50 0.026 mg/mL, 0.074% v/v, and 271 mg/mL respectively [89].

Another reports from Frenita *et al.* (2020), found that Masoyi skin essential oil contains C-10 massoialactone as the main compound, which is showed membrane disruption activity in *C. Albicans*, leading to cell death. As a quorum sensing compound for *C. Albicans*, Farnesol was observed to inhibit its growth after sample application, maybe due to insufficient cell population [90]. Also stated is that the essential oil of mayosi skin has antimicrobial activity and could inhibit the formation of *C. albicans* biofilms [91]. Bafadal (2016) suggested that masoyi oil has inhibitory activity against mono-species and multispecies biofilms of *P. aeruginosa* and *C. albicans*. Masoyi oil can inhibitory the formation and degradation of dual-species *P. aeruginosa* - *S. aureus* biofilms with MBIC50 values of 0.091% v/v and 0.012% v/v, respectively. It also provides greater inhibition of biofilm formation against *P. aeruginosa* monospecies biofilms with an MBIC50 value of 0.002% v/v while the biofilm degradation was 0.021% v/v [92].

Hamzah study showed that the C-10 massoialactone compound, the main content of masoyi, has inhibitory activity against the mono-species biofilm *E. coli*, *P. aerignosa*, and *C. albicans* at concentration 0.25% v/v and provides inhibitory activity against multispecies biofilm [4]. It also has activity against *E. coli*, *P. aerignosa*, and *C. albicans* at the concentration of 0.5% v/v. C-10 massoialactone also reduced the cell density of multispecies *E. coli*, *P. aerignosa*, and *C albicans* biofilms and damaged the EPS matrix. C-10 massoialactone compound can provide inhibitory activity above 50% in the mid-phase and maturation of *S. aureus* biofilms. It can eradicate mono - biofilm species *S. aureus*, *E. coli*, *P. aerignosa*, and *C. albicans* [83,84,93]. C-10 compounds are also reported to have inhibitory activity in the mid and maturation phases and have the ability to eradicate polymicrobial biofilms of *S. aureus*, *E. coli*, *P. aerignosa*, and *C albicans* [84]. C-10 massoialactone was also reported to have antibiofilm activity on polymicrobial catheters and can damage the polymicrobial biofilm EPS matrix [84].

4.4. Cinnamon.

Many biofilms are involved in various cases of microbial infection. Investigations have shown that *S. aureus* is the second most common cause of pathogenic bacteria found in the ICU, a significant cause of infection in women. Infection due to *S. aureus* causes more complications in treating microorganism infections in the catheter due to biofilm formation [94–96]. Among the various essential oils, plant extracts of plant origin with antimicrobial
properties are obtained from species of the genus *Cinnamomum* (*Lauraceae*) such as *Cinnamomum cassia* and *Cinnamomum zeylanicum* [97]. Essential oils are obtained from various parts of plants, with each part of different chemical composition. *C. cassia* is mainly used in various bacterial and fungal infections. The bark consists of 70 to 90% (E) -cinnamaldehyde.

![Figure 5. Molecular structure of cinnamaldehyde.](image)

**Figure 5.** Molecular structure of cinnamaldehyde.

Oou *et al.* (2006) investigated the antimicrobial activity of essential oil from *C. cassia* and cinnamaldehyde against *S. aureus*. The essential oil consists mainly of trans-cinnamaldehyde (85%) and o-methoxy-cinnamaldehyde (8.79%). The cinnamon oil exhibited a significantly less inhibitory effect against *S. aureus* (600 μg/ml) than cinnamaldehyde (250 μg/ml) [98]. Another report by Jia *et al.* (2011) investigated the antibiofilm activity of cinnamaldehyde on methicillin-resistant *S. aureus* (MRSA). The result showed that the killing effects were concentration-dependent (MIC vary from one strain to another, 0,0625-0,135 % v/v). 5x MIC concentration of cinnamaldehyde was able to detach and kill existing biofilms. The sub-MIC concentration of cinnamaldehyde could play a role in preventing biofilm formation of MRSA [99–101].

Firmino *et al.* (2018) stated that Cinnamaldehyde-rich essential oil from *Cinnamomum zeylanicum* and *Cinnamomum cassia* could inhibit the growth of gram-positive and gram-negative planktonic bacteria. It can reduce the biofilm biomass of *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* biofilm by 99%. Also, it could reduce cell viability by 5.74 Log CFU/ml. Mohamed (2018) stated found that cinnamaldehyde has antibacterial and antibiofilm activity at low concentrations against potent biofilm-producing strains (A. baumannii) at 0,875 mg/ml (MIC) and 1,75 mg/ml (MBC) [102] [103]. The mechanism of cinnamaldehyde as an antimicrobial was suggested. It can interact with cell membrane rapid inhibition of energy metabolism [104].

Friedman (2017) that *carvacrol* and *thymol* can disintegrate the cell membrane in Gram-negative bacteria (*Salmonella thphimurium*) [104]. Cinnamaldehyde could interact with cell membrane induces rapid inhibition of energy metabolism. The disruption of the proton motive forces results in leakage of small ions without the leakage of more significant components such as ATP accompanied by the inhibition of ATP generation and inhibition of membrane-bound adenosine triphosphatase (ATPase) activity. Supporting the mechanism, [105,106] cinnamaldehyde did not cause the collapse of the cell membrane but caused a profound change in the composition of the membrane's fatty acids, resulting in the alteration of its external structure. The change could facilitate the cellular incorporation of cinnamaldehyde or even other interstitial compounds.

Kot *et al.* (2019) stated that trans-Cinnamaldehyde (TC) could prevent the biofilm formation of *S. aureus*. Metabolic activity of *S. aureus* cells in biofilm and the expression levels of genes involved in the synthesis of binding factors and PIA were significantly reduced in the presence of TC at ½ MBIC already in the first-hour biofilm formation. The mechanism of action is causing a significant decrease in the expression levels of eno, ebps, and fib genes.
encoding binding proteins that may prevent the colonization of host tissues of foreign materials. TC also prevented biofilm structure formation by inhibiting the expression of genes encoding glucosamine polymer PIA involved developing multiple layers of sessile bacterial cells protected by a slime substance [107].

4. Conclusions

Biofilms related to human infection are one of many problems in managing an infectious disease. Biofilm-related infections are a growing health problem worldwide, especially for patients suffering from immune system disorders such as cancer, organ transplants, and malnutrition. There are not many antibiotics available that can effectively fight biofilm infection, which results in very high drug resistance incidence. There is an increase in infections associated with polymicrobial biofilms, including oral disease, otitis media, sinusitis, diabetic wound infections, urinary tract infections (UTIs), and cystic fibrosis. The diversity of microbes in polymicrobial biofilms can cause chronic infections that are difficult to treat compared to monomicrobial biofilms. Besides, the synergistic interactions on polymicrobial biofilms impact bacteria distribution, resulting in a change in overall biomass produced. Treatment of polymicrobial biofilm-related diseases is complex and complicated because of the presence of several infectious agents. Bacterial and fungal infections caused by biofilms are complicated to treat. 1000 times the antimicrobial dose required to kill bacteria and fungi in the form of biofilms to achieve the same results of killing planktonic cells. Infection related to polymicrobial biofilms causes additional challenges for the treatment compared to monomicrobial biofilms. The challenge is to design effective treatment strategies to inhibit microbial colonization and prevent the development of polymicrobial infections. Therefore, it is essential to look for potential new anti-polymicrobial biofilm agents obtained from plant compounds, especially in discovering new drugs.

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Conflicts of Interest

The authors declare no conflict of interest.

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