

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

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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 microbe, 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–

41]. 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].

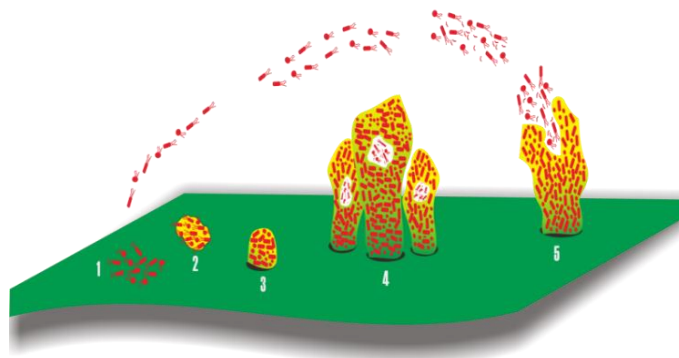


Figure 1. Biofilm Formation Process.

Hamzah *et al.* have described the biofilm formation process as stated in their paper [47]. In this paper, in line with that described by hasyru, 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 monoterpene 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].

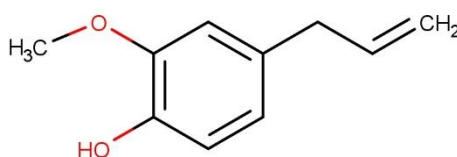


Figure 2. Molecular structure of *Eugenol*.

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. aeruginosa*, 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. aeruginosa*, 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].

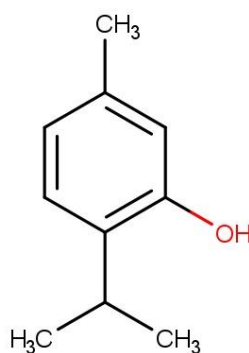


Figure 3. Molecular structure of *thymol*.

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.

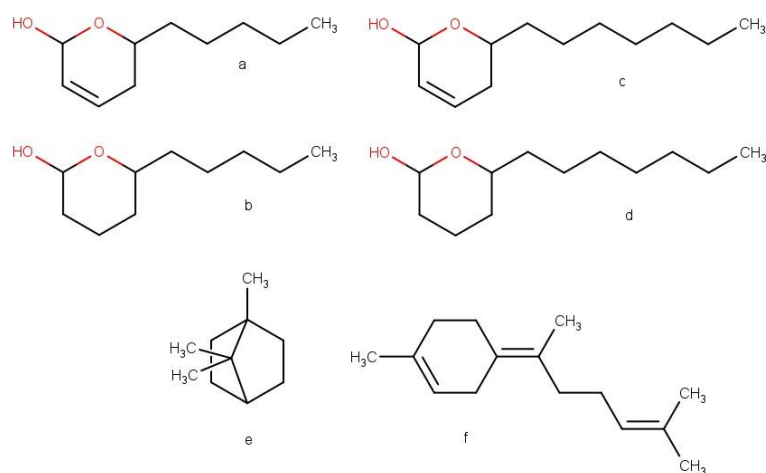


Figure 4. Several constituents of masoyi oil (a: C-10 Massoialactone, b: C-12 Massoialactone, c: δ -decalactone, d: δ -dodecalactone, e: borneol, f: β -bisabolene [78].

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 massoilactone 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 massoilactone 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.

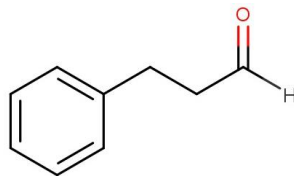


Figure 5. Molecular structure of cinnamaldehyde.

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 reports 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 thyphimurium*) [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.

References

1. Balaji, S.; Shanmugam, V.K. Enhanced Antibiofilm Activity of Endophytic Bacteria Mediated Zirconium Nanoparticles. *Biointerface Res. Appl. Chem.* **2021**, *12*, 74–82, doi:10.33263/BRIAC121.074082.
2. Grande, R.; Carradori, S. Novel Biologically Active Molecules, Biomaterials, and Nanoparticles for Microbial Biofilm Control in Human Medicine. *Molecules* **2021**, *26*, 2749, doi:10.3390/molecules26092749.
3. Muharni, M.; Ferlinahayati, F.; Yohandini, H. Antibacterial and Antibiofilm Activities of a Traditional Herbal Formula against Respiratory Infection Causing Bacteria. *Trop. J. Nat. Prod. Res.* **2020**, *4*, 527–534, doi:10.26538/tjnpr/v4i9.6.

4. Hamzah, H.; Pratiwi, S.U.T.; Hertiani, T. Efficacy of Thymol and *Eugenol* against Polymicrobial Biofilm. *Indones. J. Pharm.* **2018**, *29*, 214–221, doi:10.14499/indonesianjpharm29iss4pp221.
5. Shushanov, S.S.; Kalina, A.A.; Kravtsova, T.A.; Sherbakov, A.M.; Chernykh, Y.B.; Akentieva, N.P. Dual Effect Exhibited by Insulin in Myeloma and Lymphoblastoid Cells. *Biointerface Res. Appl. Chem.* **2021**, *12*, 2715–2728, doi:10.33263/BRIAC123.27152728.
6. Hamzah, H.; Hertiani, T.; Pratiwi, S.U.T.; Murti, Y.B.; Nuryastuti, T. The Inhibition and Degradation Activity of Demethoxycurcumin as Antibiofilm on *C. Albicans* ATCC 10231. *Res. J. Pharm. Technol.* **2020**, *13*, 377, doi:10.5958/0974-360X.2020.00075.X.
7. Samrot, A. V.; Sean, T.C. Investigating the Antioxidant and Antimicrobial Activity of Artocarpus Heterophyllus Lam . (Jackfruit) Latex. *Biointerface Res. Appl. Chem.* **2022**, *12*, 3019–3033.
8. Peters, B.M.; Jabra-Rizk, M.A.; O'May, G.A.; Costerton, J.W.; Shirtliff, M.E. Polymicrobial Interactions: Impact on Pathogenesis and Human Disease. *Clin. Microbiol. Rev.* **2012**, *25*, 193–213, doi:10.1128/CMR.00013-11.
9. Cordeiro, L.; Figueiredo, P.; Souza, H.; Sousa, A.; Andrade-Júnior, F.; Barbosa-Filho, J.; Lima, E. Antibacterial and Antibiofilm Activity of Myrtenol against Staphylococcus Aureus. *Pharmaceuticals* **2020**, *13*, 133, doi:10.3390/ph13060133.
10. Abedon, S.T.; Danis-Wlodarczyk, K.M.; Wozniak, D.J.; Sullivan, M.B. Improving Phage-Biofilm In Vitro Experimentation. *Viruses* **2021**, *13*, 1175, doi:10.3390/v13061175.
11. Azeredo, J.; Azevedo, N.F.; Briandet, R.; Cerca, N.; Coenye, T.; Costa, A.R.; Desvaux, M.; Di Bonaventura, G.; Hébraud, M.; Jaglic, Z.; et al. Critical Review on Biofilm Methods. *Crit. Rev. Microbiol.* **2017**, *43*, 313–351, doi:10.1080/1040841X.2016.1208146.
12. Wolcott, R.; Costerton, J.W.; Raoult, D.; Cutler, S.J. The Polymicrobial Nature of Biofilm Infection. *Clin. Microbiol. Infect.* **2013**, *19*, 107–112, doi:10.1111/j.1469-0691.2012.04001.x.
13. Brogden, K.A. Polymicrobial Diseases of Animals and Humans. In *Polymicrobial Diseases*; ASM Press: Washington, DC, USA, 2014; pp. 1–20.
14. Murray, J.L.; Connell, J.L.; Stacy, A.; Turner, K.H.; Whiteley, M. Mechanisms of Synergy in Polymicrobial Infections. *J. Microbiol.* **2014**, *52*, 188–199, doi:10.1007/s12275-014-4067-3.
15. Harriott, M.M.; Noverr, M.C. Ability of Candida Albicans Mutants To Induce Staphylococcus Aureus Vancomycin Resistance during Polymicrobial Biofilm Formation. *Antimicrob. Agents Chemother.* **2010**, *54*, 3746–3755, doi:10.1128/AAC.00573-10.
16. Sweidan, A.; El-Mestrah, M.; Kanaan, H.; Dandache, I.; Merhi, F.; Chokr, A. Antibacterial and Antibiofilm Activities of Scorzonera Mackmeliana. *Pak. J. Pharm. Sci.* **2020**, *33*, 199–206, doi:10.36721/PJPS.2020.33.1.REG.199-206.1.
17. Tian, X.; Schopf, A.; Amaral-Stewart, B.; Christensson, M.; Morgan-Sagastume, F.; Vincent, S.; Delatolla, R. Anammox Attachment and Biofilm Development on Surface-Modified Carriers with Planktonic- and Biofilm-Based Inoculation. *Bioresour. Technol.* **2020**, *317*, 124030, doi:10.1016/j.biortech.2020.124030.
18. Watnick, P.; Kolter, R. Biofilm, City of Microbes. *J. Bacteriol.* **2000**, *182*, 2675–2679, doi:10.1128/JB.182.10.2675-2679.2000.
19. Khatib, E.M.E.-; El-Shemy, N.S.; Ali, N.F.; Nassar, S.H. Functionalization of Natural Fibers Properties by Using TiO₂ Nanoparticles to Improve Its Antimicrobial Activity. *Biointerface Res. Appl. Chem.* **2022**, *12*, 4177–4191, doi:10.33263/BRIAC123.41774191.
20. Tartici, M.; Tartici, T.; Karaca, B.; Gür, G. Antibiofilm Activities of Fluoride Releasing Restorative Materials. *Balk. J. Dent. Med.* **2020**, *24*, 8–15, doi:10.2478/bjdm-2020-0022.
21. Petkova, N.; Hambarlyiska, I.; Tumbarski, Y.; Vrancheva, R.; Raeva, M.; Ivanov, I. Phytochemical Composition and Antimicrobial Properties of Burdock (Arctium Lappa L .) Roots Extracts. *Biointerface Res. Appl. Chem.* **2022**, *12*, 2826–2842, doi:10.33263/BRIAC123.28262842.
22. Benkhaira, N.; Koraichi, S. ibnsouda; Fikri-benbrahim, K. In Vitro Methods to Study Antioxidant and Some Biological Activities of Essential Oils : A Review. *Biointerface Reserch Appl. Chem.* **2022**, *12*, 3332–3347, doi:10.33263/BRIAC123.33323347.
23. Hamzah, H.; Hertiani, T.; Utami Tunjung Pratiwi, S.; Nuryastuti, T. The Inhibition Activity of Tannin on the Formation of Mono-Species and Polymicrobial Biofilm *Escherichia Coli*, *Staphylococcus Aureus*, *Pseudomonas Aeruginosa*, and *Candida Albicans*. *Maj. Obat Tradis.* **2019**, *24*, 110, doi:10.22146/mot.44532.
24. Nurwijayanto, A.; Na'iem, M.; Wahyuno, S.; Syahbudin, A. Screening of Antioxidants Properties from Understory Plants of Gunung Merapi National Park (Yogyakarta, Indonesia): Potential Use for Alternative Medicine. *Ecol. Environ. Conserv.* **2019**, *25*, 59–63.
25. Kusbiantoro, D.; Purwaningrum, Y. Pemanfaatan Kandungan Metabolit Sekunder Pada Tanaman Kunyit Dalam Mendukung Peningkatan Pendapatan Masyarakat Utilization of Secondary Metabolite in the Turmeric Plant to Increase Community Income. *Kultivasi* **2018**, *17*, 544–549.
26. Esmaili, F.; Sanei-Dehkordi, A.; Amoozegar, F.; Osanloo, M. A Review on the Use of Essential Oil-Based Nanoformulations in Control of Mosquitoes. *Biointerface Res. Appl. Chem.* **2021**, *11*, 12516–12529, doi:10.33263/BRIAC115.1251612529.

27. Jeong, Y.-J.; Kim, H.-E.; Han, S.-J.; Choi, J.-S. Antibacterial and Antibiofilm Activities of Cinnamon Essential Oil Nanoemulsion against Multi-Species Oral Biofilms. *Sci. Rep.* **2021**, *11*, 5911, doi:10.1038/s41598-021-85375-3.
28. Ponde, N.O.; Lortal, L.; Ramage, G.; Naglik, J.R.; Richardson, J.P. Candida Albicans Biofilms and Polymicrobial Interactions. *Crit. Rev. Microbiol.* **2021**, *47*, 91–111, doi:10.1080/1040841X.2020.1843400.
29. Achmit, M.; Aoussar, N.; Mellouki, F.; Ait Mhand, R.; Ibáñez, M.D.; Blázquez, M.A.; Akssira, M.; Zerouali, K.; Rhallabi, N. In Vitro Antibacterial and Biofilm Inhibitory Activity of the Sawdust Essential Oil of Tetraclinis Articulata (Vahl) against Catheter-Associated Staphylococcus Aureus Clinical Isolates. *Curr. Res. Biotechnol.* **2021**, *3*, 1–5, doi:10.1016/j.crbiot.2020.12.001.
30. Ncube, B.; Staden, J. Van Tilting Plant Metabolism for Improved Metabolite Biosynthesis and Enhanced Human Benefit. *Molecules* **2015**, *20*, 12698–12731, doi:10.3390/molecules200712698.
31. Liu, F.; Jin, P.; Gong, H.; Sun, Z.; Du, L.; Wang, D. Antibacterial and Antibiofilm Activities of Thyme Oil against Foodborne Multiple Antibiotics-Resistant Enterococcus Faecalis. *Poult. Sci.* **2020**, *99*, 5127–5136, doi:10.1016/j.psj.2020.06.067.
32. Fester, K.; Kutchan, T.M. Introduction to the Different Classes of Natural Products. In *Plant-derived Natural Products*; 2009; pp. 3–50.
33. Gibbons, S. An Overview of Plant Extracts as Potential Therapeutics. *Expert Opin. Ther. Pat.* **2003**, *13*, 489–497, doi:10.1517/13543776.13.4.489.
34. Guerriero, G.; Berni, R.; Muñoz-Sánchez, J.; Apone, F.; Abdel-Salam, E.; Qahtan, A.; Alatar, A.; Cantini, C.; Cai, G.; Hausman, J.-F.; et al. Production of Plant Secondary Metabolites: Examples, Tips and Suggestions for Biotechnologists. *Genes* **2018**, *9*, 309, doi:10.3390/genes9060309.
35. Hidalgo, D.; Sanchez, R.; Lalaleo, L.; Bonfill, M.; Corchete, P.; Palazon, J. Biotechnological Production of Pharmaceuticals and Biopharmaceuticals in Plant Cell and Organ Cultures. *Curr. Med. Chem.* **2018**, *25*, 3577–3596, doi:10.2174/0929867325666180309124317.
36. Ramachandra Rao, S.; Ravishankar, G.A. Plant Cell Cultures: Chemical Factories of Secondary Metabolites. *Biotechnol. Adv.* **2002**, *20*, 101–153, doi:10.1016/S0734-9750(02)00007-1.
37. Afonina, I.; Ong, J.; Chua, J.; Lu, T.; Kline, K.A. Multiplex CRISPRi System Enables the Study of Stage-Specific Biofilm Genetic Requirements in Enterococcus Faecalis. *mBio* **2020**, *11*, 1–16, doi:10.1128/mBio.01101-20.
38. Zhang, H.; Du, R.; Cao, S.; Wang, S.; Peng, Y. Mechanisms and Characteristics of Biofilm Formation via Novel DEAMOX System Based on Sequencing Biofilm Batch Reactor. *J. Biosci. Bioeng.* **2019**, *127*, 206–212, doi:10.1016/j.jbiosc.2018.07.026.
39. Donlan, R.M. Biofilm Formation: A Clinically Relevant Microbiological Process. *Clin. Infect. Dis.* **2001**, *33*, 1387–1392, doi:10.1086/322972.
40. Maione, A.; de Alteriis, E.; Carraturo, F.; Galdiero, S.; Falanga, A.; Guida, M.; Di Cosmo, A.; Maselli, V.; Galdiero, E. The Membranotropic Peptide GH625 to Combat Mixed Candida Albicans/Klebsiella Pneumoniae Biofilm: Correlation between In Vitro Anti-Biofilm Activity and In Vivo Antimicrobial Protection. *J. Fungi* **2021**, *7*, 26, doi:10.3390/jof7010026.
41. Sharma, G. CHARACTERIZATION OF ANTIMICROBIAL SUBSTANCE WITH ANTIBIOFILM ACTIVITY FROM Pediococcus Acidilactici. *J. Microbiol. Biotechnol. Food Sci.* **2020**, *9*, 979–982, doi:10.15414/jmbfs.2020.9.5.979-982.
42. Danese, P.N.; Pratt, L.A.; Kolter, R. [2] Biofilm Formation as a Developmental Process. In *Water Science and Technology*; 2001; Vol. 32, pp. 19–26.
43. Bjarnsholt, T.; Buhlin, K.; Dufrêne, Y.F.; Gomelsky, M.; Moroni, A.; Ramstedt, M.; Rumbaugh, K.P.; Schulte, T.; Sun, L.; Åkerlund, B.; et al. Biofilm Formation - What We Can Learn from Recent Developments. *J. Intern. Med.* **2018**, *284*, 332–345, doi:10.1111/joim.12782.
44. Prakash, B.; Veeregowda, B.M.; Krishnappa, G. Biofilms: A Survival Strategy of Bacteria. *Curr. Sci.* **2003**, *85*, 9–10.
45. Hamzah, H.; Hertiani, T.; Pratiwi, S.U.T.; Nuryastuti, T. Efek Saponin Terhadap Penghambatan Planktonik Dan Mono-Spesies Biofilm Candida Albicans ATCC 10231 Pada Fase Pertengahan , Pematangan Dan Degradasi. *Maj. Farm.* **2021**, *17*, 198–205, doi:10.22146/farmaseutik.v17i2.54444.
46. Wu, K.-C.; Hua, K.-F.; Yu, Y.-H.; Cheng, Y.-H.; Cheng, T.-T.; Huang, Y.-K.; Chang, H.-W.; Chen, W.-J. Antibacterial and Antibiofilm Activities of Novel Antimicrobial Peptides against Multidrug-Resistant Enterotoxigenic Escherichia Coli. *Int. J. Mol. Sci.* **2021**, *22*, 3926, doi:10.3390/ijms22083926.
47. Hamzah, H.; Hertiani, T.; Pratiwi, S.U.T.; Nuryastuti, T. Efficacy of Quercetin against Polymicrobial Biofilm on Catheters. *Res. J. Pharm. Technol.* **2020**, *13*, 5277–5282, doi:10.5958/0974-360X.2020.00923.3.
48. Murga, R.; Miller, J.M.; Donlan, R.M. Biofilm Formation by Gram-Negative Bacteria on Central Venous Catheter Connectors: Effect of Conditioning Films in a Laboratory Model. *J. Clin. Microbiol.* **2001**, *39*, 2294–2297, doi:10.1128/JCM.39.6.2294-2297.2001.

49. Gurunathan, S.; Han, J.W.; Kwon, D.-N.; Kim, J.-H. Enhanced Antibacterial and Anti-Biofilm Activities of Silver Nanoparticles against Gram-Negative and Gram-Positive Bacteria. *Nanoscale Res. Lett.* **2014**, *9*, 373, doi:10.1186/1556-276X-9-373.
50. Cepas, V.; López, Y.; Muñoz, E.; Rolo, D.; Ardanuy, C.; Martí, S.; Xercavins, M.; Horcajada, J.P.; Bosch, J.; Soto, S.M. Relationship Between Biofilm Formation and Antimicrobial Resistance in Gram-Negative Bacteria. *Microb. Drug Resist.* **2019**, *25*, 72–79, doi:10.1089/mdr.2018.0027.
51. Anderson, G.G.; Palermo, J.J.; Schilling, J.D.; Roth, R.; Heuser, J.; Hultgren, S.J. Intracellular Bacterial Biofilm-Like Pods in Urinary Tract Infections. *Science* **2003**, *301*, 105–107, doi:10.1126/science.1084550.
52. Cowan, S.E.; Gilbert, E.; Liepmann, D.; Keasling, J.D.; Bioengineering Graduate Program, J.; Francisco, S. Commensal Interactions in a Dual-Species Biofilm Exposed to Mixed Organic Compounds. *Appl. Environmental Microbiol.* **2000**, *66*, 4481–4485.
53. Leriche, V.; Briandet, R.; Carpentier, B. Ecology of Mixed Biofilms Subjected Daily to a Chlorinated Alkaline Solution: Spatial Distribution of Bacterial Species Suggests a Protective Effect of One Species to Another. *Environ. Microbiol.* **2003**, *5*, 64–71, doi:10.1046/j.1462-2920.2003.00394.x.
54. Pratiwi, S.U.T.; Hamzah, H. Inhibition and Degradation Activity of (*Sapindus Rarak* Seeds) Ethanol Extract against Polymicrobial Biofilm. *Res. J. Pharm. Technol.* **2020**, *13*, 5425–5430, doi:10.5958/0974-360X.2020.00947.6.
55. Roy, R.; Tiwari, M.; Donelli, G.; Tiwari, V. Strategies for Combating Bacterial Biofilms: A Focus on Anti-Biofilm Agents and Their Mechanisms of Action. *Virulence* **2018**, *9*, 522–554, doi:10.1080/21505594.2017.1313372.
56. Putri, R.L.; Hidayat, N.; Rahmah, N.L. Pemurnian *Eugenol* Dari Minyak Daun Cengkeh Dengan Reaktan Basa Kuat Koh Dan Ba(Oh)₂ (Kajian Konsentrasi Reaktan). *Industria* **2014**, *3*.
57. Markowitz, K.; Moynihan, M.; Liu, M.; Kim, S. Biologic Properties of *Eugenol* and Zinc Oxide-Eugenol. *Oral Surg. Oral Med. Oral Pathol.* **1992**, *73*, 729–737, doi:10.1016/0030-4220(92)90020-Q.
58. Andries, J.R.; Gunawan, P.N.; Supit, A. Uji Efek Anti Bakteri Ekstrak Bunga Cengkeh Terhadap Bakteri *Streptococcus Mutans* Secara In Vitro. *E-GIGI* **2014**, *2*, doi:10.35790/eg.2.2.2014.5763.
59. Kim, Y.; Lee, J.; Park, S.; Kim, S.; Lee, J. Inhibition of Polymicrobial Biofilm Formation by Saw Palmetto Oil, Lauric Acid and Myristic Acid. *Microb. Biotechnol.* **2021**, 1751–7915.13864, doi:10.1111/1751-7915.13864.
60. Sybiya Vasantha Packiavathy, I.A.; Agilandewari, P.; Musthafa, K.S.; Karutha Pandian, S.; Veera Ravi, A. Antibiofilm and Quorum Sensing Inhibitory Potential of *Cuminum Cuminum* and Its Secondary Metabolite Methyl *Eugenol* against Gram Negative Bacterial Pathogens. *Food Res. Int.* **2012**, *45*, 85–92, doi:10.1016/j.foodres.2011.10.022.
61. Davidson, P.M.; Taylor, T.M.; Schmidt, S.E. Chemical Preservatives and Natural Antimicrobial Compounds. In *Food Microbiology*; ASM Press: Washington, DC, USA, 2014; pp. 765–801.
62. Septiyanti, M.; Fauziyah, N.; Putri, R.; Meliana, Y. Stability Study of *Eugenol* and Kitolod Extract (*Isotoma Longiflora*) Nanoemulsion Concentrate for Botanical Pesticide Application. *Biointerface Res. Appl. Chem.* **2020**, *10*, 6389–6397, doi:10.33263/BRIAC105.63896397.
63. Marchese, A.; Barbieri, R.; Coppo, E.; Orhan, I.E.; Daglia, M.; Nabavi, S.F.; Izadi, M.; Abdollahi, M.; Nabavi, S.M.; Ajami, M. Antimicrobial Activity of *Eugenol* and Essential Oils Containing Eugenol: A Mechanistic Viewpoint. *Crit. Rev. Microbiol.* **2017**, *43*, 668–689, doi:10.1080/1040841X.2017.1295225.
64. Catherine, A.A.; Deepika, H.; Negi, P.S. Antibacterial Activity of *Eugenol* and Peppermint Oil in Model Food Systems. *J. Essent. Oil Res.* **2012**, *24*, 481–486, doi:10.1080/10412905.2012.703513.
65. Jafri, H.; Khan, M.S.A.; Ahmad, I. In Vitro Efficacy of *Eugenol* in Inhibiting Single and Mixed-Biofilms of Drug-Resistant Strains of *Candida Albicans* and *Streptococcus Mutans*. *Phytomedicine* **2019**, *54*, 206–213, doi:10.1016/j.phymed.2018.10.005.
66. Li, W.; Chen, H.; He, Z.; Han, C.; Liu, S.; Li, Y. Influence of Surfactant and Oil Composition on the Stability and Antibacterial Activity of *Eugenol* Nanoemulsions. *LWT - Food Sci. Technol.* **2015**, *62*, 39–47, doi:10.1016/j.lwt.2015.01.012.
67. Villanueva Bermejo, D.; Angelov, I.; Vicente, G.; Stateva, R.P.; Rodriguez García-Risco, M.; Reglero, G.; Ibañez, E.; Fornari, T. Extraction of Thymol from Different Varieties of Thyme Plants Using Green Solvents. *J. Sci. Food Agric.* **2015**, *95*, 2901–2907, doi:10.1002/jsfa.7031.
68. Čabarkapa, I.; Čolović, R.; Đuragić, O.; Popović, S.; Kokić, B.; Milanov, D.; Pezo, L. Anti-Biofilm Activities of Essential Oils Rich in Carvacrol and Thymol against *Salmonella Enteritidis*. *Biofouling* **2019**, *35*, 361–375, doi:10.1080/08927014.2019.1610169.
69. Dorman, H.J.D.; Deans, S.G. Antimicrobial Agents from Plants: Antibacterial Activity of Plant Volatile Oils. *J. Appl. Microbiol.* **2000**, *88*, 308–316, doi:10.1046/j.1365-2672.2000.00969.x.
70. Chatrath, A.; Gangwar, R.; Kumari, P.; Prasad, R. In Vitro Anti-Biofilm Activities of Citral and Thymol Against *Candida Tropicalis*. *J. Fungi* **2019**, *5*, 13, doi:10.3390/jof5010013.
71. Raei, P.; Pourlak, T.; Memar, M.Y.; Alizadeh, N.; Aghamali, M.; Zeinalzadeh, E.; Asgharzadeh, M.; Kafil, H.S. Thymol and Carvacrol Strongly Inhibit Biofilm Formation and Growth of Carbapenemase-Producing Gram Negative Bacilli. *Cell. Mol. Biol.* **2017**, *63*, 108, doi:10.14715/cmb/2017.63.5.20.

72. Cáceres, M.; Hidalgo, W.; Stashenko, E.; Torres, R.; Ortiz, C. Essential Oils of Aromatic Plants with Antibacterial, Anti-Biofilm and Anti-Quorum Sensing Activities against Pathogenic Bacteria. *Antibiotics* **2020**, *9*, 147, doi:10.3390/antibiotics9040147.
73. Hamzah, H.; Rasdianah, N.; Nurwijayanto, N.; Nandini, E. Aktivitas Ekstrak Etanol Daun Calincing Terhadap Biofilm *Candida Albicans*. *J. Farmasetis* **2021**, *10*, 21–28.
74. Oliveira, T.N.S.; Silva-Filho, C.M.S.; Malveira, E.A.; Aguiar, T.K.B.; Santos, H.S.; Albuquerque, C.C.; Morais, M.B.; Teixeira, E.H.; Vasconcelos, M.A. Antifungal and Antibiofilm Activities of the Essential Oil of Leaves from *Lippia Gracilis* Schauer against Phytopathogenic Fungi. *J. Appl. Microbiol.* **2021**, *130*, 1117–1129, doi:10.1111/jam.14857.
75. Mulvey, M.A. Adhesion and Entry of Uropathogenic *Escherichia Coli*. *Cell. Microbiol.* **2002**, *4*, 257–271, doi:10.1046/j.1462-5822.2002.00193.x.
76. Wiles, T.J.; Kulesus, R.R.; Mulvey, M.A. Origins and Virulence Mechanisms of Uropathogenic *Escherichia Coli*. *Exp. Mol. Pathol.* **2008**, *85*, 11–19, doi:10.1016/j.yexmp.2008.03.007.
77. Lee, J. -H.; Kim, Y. -G.; Lee, J. Carvacrol-rich Oregano Oil and Thymol-rich Thyme Red Oil Inhibit Biofilm Formation and the Virulence of Uropathogenic *Escherichia Coli*. *J. Appl. Microbiol.* **2017**, *123*, 1420–1428, doi:10.1111/jam.13602.
78. Inhalation Effect of Massoialactone from *Massoia* Essential Oil on Lipid Profile, Liver Tissues, and Body Weight of Sprague Dawley Rat. *J. Appl. Pharm. Sci.* **2019**, *9*, 111–116, doi:10.7324/JAPS.2019.90815.
79. Rollando, R. Uji Antimikroba Minyak Atsiri Masoyi (*Massoia Aromatica*) TERHADAP BAKTERI *Streptococcus Mutans*. *Maj. Farm. Dan Farmakol.* **2019**, *23*, 52–57, doi:10.20956/mff.v23i2.6585.
80. Iskandar, I.; Ismanto, A. Tinjauan Beberapa Sifat Dan Manfaat Tumbuhan Masoyi (*Massoia Aromaticum* Becc.). *War. Tumbuh. Obat Indones.* **1999**, *5*.
81. Young, R.; Rodney, T. *Essential Oil Safety*; Elsevier, 2014; ISBN 9780443062414.
82. Nawangningrum, D.; Widodo, S.; Suparta, I.M.; Holil, M. Kajian Terhadap Naskah Kuna Nusantara Koleksi Fakultas Ilmu Pengetahuan Budaya Universitas Indonesia: Penyakit Dan Pengobatan Ramuan Tradisional. *Makara Seri Sos.* **2004**, *8*, 45–53, doi:10.7454/mssh.v8i2.86.
83. Rali, T.; Wossa, S.; Leach, D. Comparative Chemical Analysis of the Essential Oil Constituents in the Bark, Heartwood and Fruits of *Cryptocarya Masoy* (Oken) Kosterm. (Lauraceae) from Papua New Guinea. *Molecules* **2007**, *12*, 149–154, doi:10.3390/12020149.
84. Hamzah, H.; Hertiani, T.; Pratiwi, S.U.T.; Nuryastuti, T.; Gani, A.P. Antibiofilm Studies of Zerumbone against Polymicrobial Biofilms of *Staphylococcus Aureus*, *Escherichia Coli*, *Pseudomonas Aeruginosa*, and *Candida Albicans*. *Int. J. Pharm. Res.* **2020**, *12*, 1307–1314, doi:10.31838/ijpr/2020.SP1.211.
85. Barros, M.E.S.B.; Freitas, J.C.R.; Oliveira, J.M.; da Cruz, C.H.B.; da Silva, P.B.N.; de Araújo, L.C.C.; Militão, G.C.G.; da Silva, T.G.; Oliveira, R.A.; Menezes, P.H. Synthesis and Evaluation of (–)-Massoialactone and Analogues as Potential Anticancer and Anti-Inflammatory Agents. *Eur. J. Med. Chem.* **2014**, *76*, 291–300, doi:10.1016/j.ejmech.2014.02.013.
86. Pratiwi, S.U.T.; Lagendijk, E.L.; Hertiani, T.; De Weert, S.; Cornellijs, A.M.; Van Den Hondel, J.J. Antimicrobial Effects of Indonesian Medicinal Plants Extracts on Planktonic and Biofilm Growth of *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*. *Int. J. Pharm. Pharm. Sci.* **2015**, *7*, 183–191, doi:10.4172/2376-0354.1000119.
87. Hertiani, T.; Yuswanto, A.; Utami Tunjung Pratiwi, S.; Muthma'innah Mashar, H. Effect of *Massoia* (*Massoia Aromatica* Becc.) Bark on the Phagocytic Activity of Wistar Rat Macrophages. *Sci. Pharm.* **2018**, *86*, 19, doi:10.3390/scipharm86020019.
88. Nuță, D.C.; Limban, C.; Chiriță, C.; Chifiriuc, M.C.; Costea, T.; Ioniță, P.; Nicolau, I.; Zarafu, I. Contribution of Essential Oils to the Fight against Microbial Biofilms—A Review. *Processes* **2021**, *9*, 537, doi:10.3390/pr9030537.
89. Hertiani, T.; Pratiwi, S.; Yuswanto, A.; Permanasari, P. Potency of *Massoia* Bark in Combating Immunosuppressed-Related Infection. *Pharmacogn. Mag.* **2016**, *12*, 363, doi:10.4103/0973-1296.185771.
90. Triana Hertiani; Frenita Burhan; Mentarry Bafadal; Sylvia Utami Tunjung Pratiwi Membrane Cell Disruption of *Candida Albicans* by Masoyi Bark Essential Oil. *Int. J. Res. Pharm. Sci.* **2020**, *11*, 2598–2602, doi:10.26452/ijrps.v11i2.2268.
91. Hidayah, N.; Mustafa, H.; Murni, M.; Tolistiawaty, I. Efektifitas Repelan Losion Minyak Atsiri Kulit Jeruk Bali (*Citrus Maxima* (Burm.) Merr.) Terhadap *Aedes Aegypti*. *BALABA J. LITBANG Pengendali. PENYAKIT BERSUMBER BINATANG BANJARNEGARA* **2018**, 159–168, doi:10.22435/blb.v14i2.403.
92. Bafada, M. Efek Minyak Masoyi (*Massoia Aromatica* Becc.) Terhadap Kultur Multispesies Biofilm, Universitas Gadjah Mada, 2016.
93. Utami, D.; Tunjung Pratiwi, S.; Spaink, H.; Haniastuti, T.; Hertiani, T. Antibiofilm Effect of C-10 *Massoia* Lactone toward Polymicrobial Oral Biofilms. *J. Adv. Pharm. Technol. Res.* **2021**, *12*, 89, doi:10.4103/japtr.JAPTR_105_20.
94. Al-Shuneigat, J.; Al-Sarayreh, S.; Al-Saraireh, Y.; Al-Qudah, M. Antibacterial and Antibiofilm Activity of Essential Oil of *Achillea Biebersteinii* and Its Mode of Action. *Curr. Issues Pharm. Med. Sci.* **2020**, *33*, 83–89, doi:10.2478/cipms-2020-0016.

95. Nabavi, S.; Di Lorenzo, A.; Izadi, M.; Sobarzo-Sánchez, E.; Daglia, M.; Nabavi, S. Antibacterial Effects of Cinnamon: From Farm to Food, Cosmetic and Pharmaceutical Industries. *Nutrients* **2015**, *7*, 7729–7748, doi:10.3390/nu7095359.
96. Vasconcelos, N.G.; Croda, J.; Simionatto, S. Antibacterial Mechanisms of Cinnamon and Its Constituents: A Review. *Microb. Pathog.* **2018**, *120*, 198–203, doi:10.1016/j.micpath.2018.04.036.
97. Doyle, A.A.; Stephens, J.C. A Review of Cinnamaldehyde and Its Derivatives as Antibacterial Agents. *Fitoterapia* **2019**, *139*, 104405, doi:10.1016/j.fitote.2019.104405.
98. Ooi, L.S.M.; Li, Y.; Kam, S.-L.; Wang, H.; Wong, E.Y.L.; Ooi, V.E.C. Antimicrobial Activities of Cinnamon Oil and Cinnamaldehyde from the Chinese Medicinal Herb Cinnamomum Cassia Blume. *Am. J. Chin. Med.* **2006**, *34*, 511–522, doi:10.1142/S0192415X06004041.
99. Shan, B.; Cai, Y.-Z.; Brooks, J.D.; Corke, H. Antibacterial Properties and Major Bioactive Components of Cinnamon Stick (Cinnamomum Burmannii): Activity against Foodborne Pathogenic Bacteria. *J. Agric. Food Chem.* **2007**, *55*, 5484–5490, doi:10.1021/jf070424d.
100. Jia, P.; Xue, Y.J.; Duan, X.J.; Shao, S.H. Effect of Cinnamaldehyde on Biofilm Formation and SarA Expression by Methicillin-Resistant Staphylococcus Aureus. *Lett. Appl. Microbiol.* **2011**, *53*, 409–416, doi:10.1111/j.1472-765X.2011.03122.x.
101. Subash Babu, P.; Prabuseenivasan, S.; Ignacimuthu, S. Cinnamaldehyde—A Potential Antidiabetic Agent. *Phytomedicine* **2007**, *14*, 15–22, doi:10.1016/j.phymed.2006.11.005.
102. Firmino, D.F.; Cavalcante, T.T.A.; Gomes, G.A.; Firmino, N.C.S.; Rosa, L.D.; de Carvalho, M.G.; Catunda Jr, F.E.A. Antibacterial and Antibiofilm Activities of Cinnamomum Sp. Essential Oil and Cinnamaldehyde: Antimicrobial Activities. *Sci. World J.* **2018**, *2018*, 1–9, doi:10.1155/2018/7405736.
103. Antibacterial and Antibiofilm Activity of Cinnamaldehyde against Carbapenem-Resistant Acinetobacter Baumannii in Egypt: In Vitro Study. *J. Appl. Pharm. Sci.* **2018**, *8*, 151–156, doi:10.7324/JAPS.2018.81121.
104. Friedman, M. Chemistry, Antimicrobial Mechanisms, and Antibiotic Activities of Cinnamaldehyde against Pathogenic Bacteria in Animal Feeds and Human Foods. *J. Agric. Food Chem.* **2017**, *65*, 10406–10423, doi:10.1021/acs.jafc.7b04344.
105. Di Pasqua, R.; Betts, G.; Hoskins, N.; Edwards, M.; Ercolini, D.; Mauriello, G. Membrane Toxicity of Antimicrobial Compounds from Essential Oils. *J. Agric. Food Chem.* **2007**, *55*, 4863–4870, doi:10.1021/jf0636465.
106. Helander, I.M.; Alakomi, H.-L.; Latva-Kala, K.; Mattila-Sandholm, T.; Pol, I.; Smid, E.J.; Gorris, L.G.M.; von Wright, A. Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. *J. Agric. Food Chem.* **1998**, *46*, 3590–3595, doi:10.1021/jf980154m.
107. Kot, B.; Sytykiewicz, H.; Sprawka, I.; Witeska, M. Effect of Trans-Cinnamaldehyde on Methicillin-Resistant Staphylococcus Aureus Biofilm Formation: Metabolic Activity Assessment and Analysis of the Biofilm-Associated Genes Expression. *Int. J. Mol. Sci.* **2019**, *21*, 102, doi:10.3390/ijms21010102.