ANTIMICROBIAL ACTIVITY OF BIODEGRADABLE PLASTIC BASED ON CORNCOB WASTE INCORPORATED WITH CLOVE ESSENTIAL OIL

Ari Susanti[1], Dhea Kana Zafira[2]
ari.susanti@polinema.ac.id[1], dheakana82@gmail.com[2]
Politeknik Negeri Malang[1], Universitas Jember[2]

ABSTRAK

Jagung (Zea mays L.) merupakan komoditi tanaman pangan penting yang mempunyai nilai strategis serta peran kedua di Indonesia. Permintaan konsumen yang tinggi akan makanan segar dan globalisasi pasar dunia memungkinkan perdagangan komoditas berkembang lebih efisien dengan menggunakan kemasan yang memperpanjang umur simpan. Tongkol jagung merupakan limbah dengan kandungan selulosa yang tinggi untuk menghasilkan plastik biodegradable sebagai kemasan yang ramah lingkungan. Minyak atsiri cengkeh mengandung eugenol yang tinggi untuk meningkatkan ketahanan plastik biodegradable. Tahap awal dalam penelitian ini adalah isolasi selulosa tongkol jagung, dilanjutkan dengan pembuatan film dan pengamatan aktivitas penghambatan bakteri dengan metode spread plate. Plastik biodegradable diformulasikan dengan bahan dasar selulosa tongkol jagung dan minyak atsiri cengkeh dengan konsentrasi 0% dan 1%. Penghambatan zona aktivitas antimikroba yang lebih baik diamati dengan penambahan minyak atsiri cengkeh. Indeks daya hambat menunjukkan adanya penghambatan bakteri pada plastik biodegradable. Indeks rata-rata zona hambat 3.237 dan 2.617 menunjukkan daya hambat film terhadap Escherichia coli. Sedangkan indeks zona hambat 1.000 dan 1.071 menunjukkan daya hambat film terhadap Streptococcus mutans.
Kata Kunci : aktivitas penghambatan bakteri; makanan segar; minyak essensial oil cengkeh; plastik biodegradable; tongkol jagung.

ABSTRACT

Corn (Zea mays L.) is an essential food crop commodity with a strategic value and second role in Indonesia. High consumer demand for fresh food and the globalization of the world market enables the commodity trade to develop more efficiently by using packaging that extends the shelf life. Corncob is a waste with a high cellulose content to produce biodegradable plastic as environmentally friendly packaging. Clove essential oil contains high eugenol to increase the resistance of biodegradable plastic. The early stage in this study was the isolation of corncob cellulose, followed by the preparation of film and observation of bacterial inhibitory activity by the spread plate method. Biodegradable plastic was formulated with a corncob cellulose base and clove essential oil concentrations of 0% and 1%. The addition of clove essential oil improved the suppression of the antibacterial activity zone. The inhibition index measures bacteria inhibition on biodegradable polymers. The average inhibition zone index of 3.237 and 2.617 revealed that the film was inhibiting Escherichia coli. Meanwhile, the inhibition zone indexes of 1.000 and 1.071 suggested that the film was inhibited against Streptococcus mutans.
Keywords : *bacterial inhibitory activity; biodegradable plastic; clove essential oil; corncob; fresh food*

INTRODUCTION

The increasing population growth in Indonesia has a direct impact on the high demand for food consumption, especially fresh food such as meat, which is predicted to expand by 29.55 kg/per capita/year in 2024; vegetables by 58.35 kg/per capita/year; and fish by 29.55 kg/capita/year. Fresh food was highly perishable, and food loss and waste account for almost one-third of worldwide food production, with losses occurring at the production stage by 24-30%, post-harvest by 20%, and consumption by 30-35% (BPK, 2019). Improved packaging that allows for less misplacement or waste every year due to shelf-life expiration, change, or spoiling due to microbiological recreation is required. Antimicrobial bio-based packaging is one of the promised concepts for extending the shelf life of sparkling meals to prevent bacterial growth, ensuring improved quality and safety for sparkling food. Bio-based packaging has developed as a viable alternative to traditional plastics derived from petroleum, which cause major environmental difficulties and contribute to material recovery and renewable resource exploitation (Hazrol et al., 2021). The long-term presence of plastics in the environment, as well as their resistance to disintegration, led to the development of other polymers, such as biodegradable plastic. Biodegradable plastic is one of the potentials environmentally friendly packaging that can help to alleviate current environmental challenges such as the negative impact of non-biodegradable petroleum-based plastics, the use of plant waste, and the rise of waste mountains. Plant-based fibres made of cellulose, hemicellulose, and lignin have been proposed as biopolymer sources for producing bioplastics that can replace traditional reinforce fibres (glass, carbon, and kevlar) (Hazrol et al., 2021). Natural fibres were chosen because of their non-toxicity, lower cost, and ease of breakdown by microbes.

Corn (Zea Mays L.) is the most widely grown crop in the world and a good source of plant leftovers, with benefits such as high starch levels and biodegradability. In Indonesia, total corn production is roughly 54,74 quintals/hectare, with waste corncob accounting for approximately 20% of total corn production (Syafiq et al., 2020). Corn cobs are the by-product of extracting maize kernels from the fruit. Corncob also has a high cellulose concentration of about 45% and is a sustainable source due to its abundance. Corn cobs have a high cellulose content, making them a natural supply of cellulose with the potential to be employed as disintegrating nanocomposite films for food packaging (Sinaga et al., 2018).

Antimicrobial applications are among the exceptional techniques for actively managing bacterial and fungal proliferation that causes meal deterioration and prevents the growth of microorganisms, including pathogenic microorganisms (food safety) (Yunus & Fauzan, 2019). Consumers are increasingly interested in the usage of herbal antimicrobial components in packaging as they become more aware of the potentially harmful effects of synthetic preservatives on health. The Food and Drug Administration has classified essential oils (EOs) as GRAS (Generally Recognized as Safe) food components. EOs derived from leaves, flowers, fruits, seeds, rhizomes, roots, and bark of cloves, cinnamon, thyme, ginger, oregano, rosemary, garlic, and other plants. Spices with essential oils have antibacterial effects, making them exciting food additions (Hasanuddin & Subakir, 2020). Because of their low microbial resistance, bioplastics have been limited in their application.

As antimicrobial packaging, biodegradable plastic integrated with essential oils is being created and explored, and it has shown that the use of small concentrations of EOs can improve mechanical properties, allowing the film to hold its shape for a longer period of time (Syafiq et al., 2020). The addition of antimicrobial agents is more useful on the food surface (packing) because to their controlled release rather than immediate assimilation into the meals (Sánchez-González et al., 2011). Because it contains high eugenol (70-80%), oleic acid, and lipids as antiseptic, antimicrobial, antibacterial, antifungal, anticarcinogenic, and anti-free radicals, essential oil from cloves may also be used as an antimicrobial agent as a mixture in the manufacture of biodegradable plastics (Sinaga et al., 2018). Clove EOs' ability to denature proteins and react with phospholipid cell membranes influences the suppression of numerous gram-negative and gram-positive bacteria (Yunus & Fauzan, 2019). The goal of this study was to create bioplastics from corncob waste that have the antibacterial potential to restrain or prevent the growth of spoilage and pathogenic microorganisms like Escherichia coli (gram-negative bacteria) and Streptococcus mutans (gram-positive bacteria).

RESEARCH METHODS

1. Materials

The raw material for this study is cellulose, which was gathered from local farmers in Jember Regency, East Java. Tapioca flour was used to make the starch. In addition, Sumber Atsiri shop in Cilacap provided the NaOH (5%), distilled water, NaOCl (2%), HNO3 (2%), H2O2 (2%), glycerol (99%), and Clove EOs.

2. Isolation of Corn Cob Cellulose

The bleaching process was used to isolate cellulose from corn cob. Corn cob powder (particle size 80 mesh) was added to NaOH solution in a 1:40 (w/v) ratio and stirred continuously for 1 hour. After settling for two hours, the residue was collected. The residue was dissolved in 180 ml NaOCl solution and heated for 1 hour with a thermal-magnetic combination to 70°C, followed by filtration. The residue was collected and mixed with HNO3 in a 1:2 ratio at 80°C for an hour. This technique was repeated twice, and the residue was dried in an oven until corncob cellulose powder was obtained.

3. Film Preparation

The components cellulose powder, aquadest, starch, and glycerol were combined in the following proportions: 1: 92.5: 5: 1 (w/v%). Two samples were made with varied quantities of clove EOs, 0% and 1% (v/v). The needed amount of composition was added to a beaker and swirled at 400 rpm for 1 hour at room temperature to synthesis the bioplastic samples. The film-making solution was poured into a glass mold lined with five sheets of aluminum foil. The produced films were gently removed from the glass mold and sliced into 4 cm2 bioplastic samples.

4. Antimicrobial Analysis

Initially, the disk diffusion method was employed to assess the antibacterial characteristics of bioplastics. Previously, the inhibitory power of bacteria was investigated using two types of bacteria: Escherichia Coli (gram-negative bacteria) and Streptococcus mutans (gram-positive bacteria). Each bacterium was taken in tons of 1 Osse and then placed in roughly 10 mL of media (inoculum NB). The bacterium pre-culture was cultured at room temperature for 24 hours with mild shaking. Film samples (2 cm × 2 cm) were placed on sterilized plates, and 100 L of bacterial lifestyle was added before flattening on the test medium. Bacterial cultures were incubated twice a day for 24 hours. Following this, the clean zone was examined and its diameter was determined. For each measurement, at least three replicates were performed. The generated clean zone demonstrated the ability to suppress microorganisms. Digital vernier callipers are used to measure the diameters of an inhibiting zone (clear zone) surrounding it. The inhibitory zone index was obtained using total diameter – flat diameter.

FINDING

The bleaching process is used to isolate cellulose in this investigation. Then, using clove essential oil, create a biodegradable plastic based on maize cobs. Bacteria Escherichia coli and Streptococcus mutans were cultivated on inoculum NB using the spread plate method. The disk diffusion method was used to investigate the influence of clove essential oil on the antibacterial activity of biodegradable plastic against Escherichia coli (gram-negative) and Streptococcus mutans (gram-positive). The transparent quarter form demonstrated microbe suppression, often known as antibacterial activity. Within three repetitions dan two treatment called A as a control for added 0% EOs and B for added 1% EOs, the data below demonstrated the antibacterial activity of biodegradable plastic based on corncob combined with clove EOs. The film inhibition test was performed against Escherichia coli bacteria, and the greatest index inhibition zone was 2.733 on second replica for 1% EOs composition compared with control.

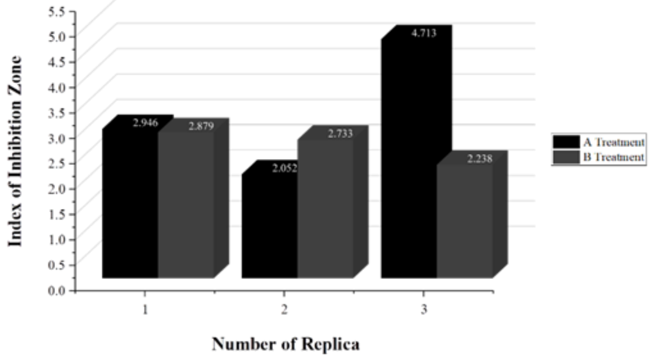

Figure 1. Index Inhibition
Zone Against Escherichia coli

According to these data, the addition of 1% clove EOs has a marginally significant effect on Escherichia coli growth suppression. Clove essential oils' high eugenol content acts as an antibacterial agent. Because eugenol is hydrophobic, it can partition with the lipids found in bacteria and mitochondrial cell membranes, making them more permeable by altering cell structures. This results in considerable leaking of vital chemicals and ions from the bacterial cell, which ultimately leads to cell death (Jeyakumar & Lawrence, 2021). Antimicrobial research from the addition of clove EOs to biodegradable plastic revealed that the Corncob-Clove EOs combo movie had no specific inhibitory effect on the development of Streptococcus mutans. The clear or inhibitory region was previously overlooked.

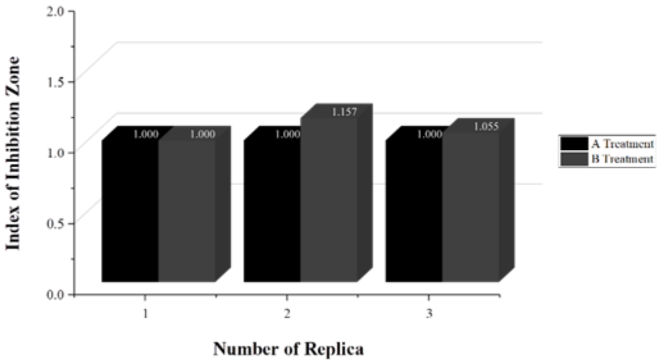

Figure 2. Index Inhibition
Zone Against Streptococcus mutans

The maximum index inhibition zone of Streptococcus mutans bacteria was only 1.157 within 1% Clove EOs film composite compared with control. The final active hydrophobic compounds in the corncob-EOs mixture motion pictures were insufficient to cause Streptococcus mutans bacteria phone lysis, resulting in little inhibition of bacteria growth (Hasanuddin & Subakir, 2020). Because the phone wall structure of gram-negative bacteria is thinner than that of gram-positive microorganisms, antimicrobial agents can more easily penetrate cell membranes and harm cell membranes of gram-negative microorganisms (Putra et al., 2017). Furthermore, the outer membrane of gram-negative bacteria (Escherichia coli) is constituted mostly of lipopolysaccharides, whereas the outer membrane of gram-positive bacteria (Streptococcus mutans) is composed primarily of peptidoglycan connected with polysaccharides and teichoic acids (Misra et al., 2014). Eugenol penetrates lipopolysaccharides more easily and changes the structure of the cell wall. The changing structure of the cell wall causes intracellular leakage and has an impact on bacterial development. The eugenol-induced leakage of intracellular material in gram-negative bacteria is larger than that reported in gram-positive bacteria, resulting in more bacterial cell death in Escherichia coli (gram-negative bacteria) than Streptococcus mutans (gram-positive bacteria) (Jeyakumar & Lawrence, 2021).

Figure 3. Inhibition Zone
Against Escherichia coli

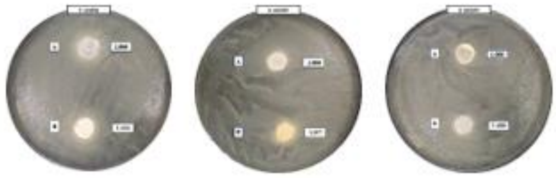

Figure 4. Inhibition Zone
Against Streptococcus mutans

1. Technique to Evaluate Antimicrobial Activity of Film Incorporated Clove Essential Oils

Clove EOs had a significant impact on biodegradable plastic features by increasing bacterial inhibition against Escherichia coli, similar to the examination of edible film carboxyethyl cellulose in collaboration with clove EOs using the agar disk diffusion method. The disk diffusion technique was formerly simple and low-cost, capable of testing a large variety of bacteria and antimicrobial compounds, and easy to understand the results. Film samples were cut into discs with a 10 mm diameter and placed on MHA plates that had previously been covered with 100 L of broth cultures containing roughly 108 CFU/mL of various food pathogens such as Escherichia coli. The plates were incubated at 37°C for 24 hours. This search resulted in an increase in EOs attention from 1% to 3%, as well as an increase in inhabitation area from 14,66 mm to 24,33 mm (Dashipour et al., 2014).

A good diffusion method was another approach frequently utilized to investigate antibacterial activity of plant life or microbial extracts. The extract solution is packed into a hole or well produced on the agar medium in the diffusion method, whereas the agar disc diffusion method sets a filter paper disc holding the test solution on the agar surface (Balouiri et al., 2016). Film-based potato starch mixed with clove oil EOs shown antibacterial action (Singh et al., 2018). The mechanism of the antimicrobial test was a well cut on solid nutritional agar media with a diameter of 10 mm that was infected with 0.1 ml Escherichia coli bacteria in the range of 10-100 CFU/ml and then incubated at 37oC for 24 hours. Clove oil was found to be more effective than peppermint oil at inhibiting microorganisms such as Escherichia coli in this study. The clear zone of resistance to Escherichia coli microbiological of the film potato starch with clove EOs was 17.89 mm, which was higher than the clear zone of resistance to Escherichia coli microbial of the film potato starch with peppermint oil, which was only 15.28 mm.

The diffusion method determined the minimum inhibitory concentration (MIC), or the lowest level of antibacterial agent in clove EOs that inhibits organism development in tubes or microdilution wells as recognized by naked eye. Through an agar nicely diffusion test, the average MIC suitable for eating film constructed entirely on corn starch and employing clove oil as an antibacterial agent was 0.49 mg/ml against Escherichia coli. A microbiological suspension was created using an unfold plate approach, inoculated and put into a petri dish, then wells with a diameter of 6 mm were created and packed with extraordinary concentrations of clove oil, incubated at acceptable settings (Natania & Setiawan, 2020).

Total plate count was used to test the efficacy of an edible film based on carrageenan and clove essential oils applied on fresh Sardinella lemurs. The total plate count approach could be used to describe the influence of edible film containing clove essential oils on the shelf life of fresh fish. The TPC test on edible film treated with clove EOs yielded a reduced total bacterial value of 9.4 x 102 colonies/gr compared to edible film not treated with clove EOs, which yielded 1.4 x 103 colonies/gr. According to the findings of this study, adding active antibacterial agents to edible film has an advantage in terms of increasing the shelf life of fresh food (Jaja et al., 2021).

2. Antimicrobial Properties of Film Incorporated with Clove Essential Oils

Clove essential oils are effective against all check microorganisms, resulting in a wide range of antibacterial effects. Clove EOs is likely to be antimicrobial agents, serving an important purpose in the packaging industry by inhibiting microbe growth. Thus, many researchers are interested in learning more about its effect on both gram-negative and gram-positive bacteria. The gram-negative bacteria employed in this study was Escherichia coli, and clove EOs increased the inhibitory index region with 1% addition.

Many research on the antibacterial properties of films containing clove EOs have previously been conducted against other gram-negative bacteria that commonly contaminate fresh food, such as S. Typhimurium and L. monocytogenes. L. monocytogenes is a pathogenic bacterium that causes listeriosis in dairy and meat products, whereas Salmonella Typhimurium infection causes gastroenteritis in humans. Previous research investigated the effect of clove EOs on S. Typhimurium and L. monocytogenes, as well as other gram-negative bacteria, and the film's improved antibacterial action. There was no inhibitory zone identified in alginate bio composite films devoid of essential oil (control film). The addition of clove EOs to alginate bio composite films was particularly effective against S. Typhimurium, exhibiting a large clear region (inhibition zone) up to 65 mm in and inhibiting L. monocytogenes up to 29 mm concentration. Eos clove 40% (Frank et al., 2018). Similarly, with suitable for eating apple film integrated clove EOs performed even at the lowest concentration used in the film formulation, L. monocytogenes growth should be prevented by the use of 1.5% - 3% clove oil concentration until the 27 mm perimetral inhibitory zone. Other research on biodegradable plastic antimicrobials has shown that clove EOs inhibit the growth of some microorganisms commonly associated with the food industry, with inhibition diameters of 12 to 18.5 mm for E. coli and 6 to 14 mm for L. monocytogenes at concentrations of 0.1 - 0.5% Eos (Du et al., 2009) (Moritz et al., 2020).

The antibacterial activity of biodegradable plastic based on corncob-incorporated clove EOs has an impact on the development of Streptococcus mutans, gram-positive bacteria in this study, however it is not significant. Slightly inhibition regions were for this film composition corncob with clove EOs. This result differed significantly from previous research conducted by (Frank et al., 2018) where the inhibition zone was roughly 19.67 to 37.33 mm with an extra concentration of 20%-40 in film (v/w). This could be owing to the lack of eugenol as an antibacterial agent in this video. Eugenol has the potential to depolarize the bacterium membrane and disrupt the cytoplasmic membrane, ultimately leading to cell death (Jeyakumar & Lawrence, 2021).

Although clove EOs did not significantly affect S. Mutans in this study, clove EOs demonstrated antimicrobial activity in films via different gram-positive microorganism such as Staphylococcus aureus, B. cereus verified suitable for eating starch movies containing various concentrations of oils of clove and screened its inhibition recreation in opposition to Staphylococcus aureus and B. cereus discovered that the zones of inhibition for the microorganism improved (G. Al-Hashimi et al., 2020).

To combat microbiological activity, several antimicrobial EOs additives and concentrations on increasing biodegradable packaging were studied and applied to the composite film. According to the findings of this investigation, adding EOs to a biodegradable film composite improved the film's ability to prevent the growth of bacteria throughout positive-gram and negative-gram bacteria. According to one study, essential oils (thyme and clove) were mixed throughout PLA-PBAT biodegradable films and examined for their ability to suppress the bacteria Escherichia coli. The thyme oil composite film (1 wt.%) decreased E. coli growth by only 55.96%, but the clove oil composite film (1% wt.) prevented E. coli growth by 60.31%. When the concentration was raised to 10% wt., more inhibition was seen, and the oil composite for clove was around 93.43%. This is because clove oil contains a larger percentage of eugenol than thyme oil, both of which operate as antibacterial agents. Clove EOs were found to be more capable of preventing microorganism development than film-thyme Eos (Sharma et al., 2020).

In another study, assessments of the effectiveness of suitable for eating film containing clove EOs and cinnamon EOs revealed that at the same dose (6%). A improved understanding of EOs results in greater suppression of fungus (Aspergillus flavus) and gram-positive microorganisms (S.aureus and Penicillium sp). Film-clove EOs have been shown to have an excellent influence on microorganisms in low concentrations, resulting in better suppression of halos bacteria in dried fish (Matan, 2012). Other research reveals that lemon essential oils have an effect on the physiological characteristics and antibacterial activity of corn and wheat starch films. The antibacterial activity study revealed that a greater awareness of EOs has a stronger inhibition against Escherichia coli (gram-negative bacteria) and S.aureus (gram-positive bacteria). There is no microorganism inhibiting zone for movie disk wit (Song et al., 2018).

CONCLUSION

To reduce environmental risks and enhance the shelf life of fresh food products, an antimicrobial biodegradable plastic made from corncob and infused with clove essential oils (EOs) can be used instead of standard packaging. The addition of clove oil changed the characteristics of the composite film, increasing its antibacterial efficacy against various harmful microorganisms common in fresh food. Clove EOs-corncob composite film reduced Escherichia coli growth when 1% wt. clove oil was used. Clove EOs-corncob-based films are environmentally friendly, abundant natural resources in Indonesia, and have antibacterial qualities.

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