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Optimization of Chitosan-Based Edible Film with the Addition of Velvet Bean Aqueous Extract and Cinnamon Essential Oil for Antibacterial Packaging

Sri Retno Dwi Ariani[1]*, Khoirur Rohmaniatush Sholihah[1], Endang Susilowati[1], Widiastuti Agustina Eko Setyowati[1], Ifah Munifah[2], Tyas Dwi Rohmatun[1], Nadia Indri Safitri[1]

[1]Chemistry Education Study Program, Faculty of Teacher Training and Education, Universitas Sebelas Maret, Surakarta, Central Java 57126, Indonesia
[2]Research Center for Marine and Land Bioindustry, National Research and Innovation Agency (BRIN), Lombok 83352, Indonesia

*Correspondence author email: sriretno71@staff.uns.ac.id

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ABSTRACT. The current global warming issue has encouraged a lot of research on edible films. The use of edible films has become an international trend and is now essential for our products to compete in the global market. Bioactive compounds from plants can be incorporated into edible films to enhance their biological activity, including their antibacterial properties. This study developed a chitosan-based edible film with the addition of velvet bean aqueous extract (VAE) and cinnamon essential oil (CEO), aiming for high antibacterial activity. The objectives of this study were to isolate and identify the chemical components of CEO, test its antibacterial activity, find the most optimum formulation of a chitosan-based edible film with VAE and CEO in terms of antibacterial activity (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923), and analyze the physical and morphological properties of the resulting edible film. The research methods included a literature review and laboratory experiments, with procedures involving the isolation and GC-MS-based identification of CEO chemical components, antibacterial activity testing of CEO, optimization of a chitosan-based edible film formulation with the addition of VAE and CEO based on antibacterial activity test against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 (well diffusion method), and characterization of the best film's physical properties (thickness, tensile strength, elongation, solubility, WVP, and WVTR) and morphological analysis (SEM method). The results of this study were as follows: (a) CEO contained cinnamaldehyde (52.86%) and 2- methoxycinnamaldehyde (47.06%); (b) CEO had very strong antibacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, with inhibition zones of 46.28 mm and 47.95 mm, respectively; (c) the most optimal edible film formulation consisted of chitosan with 30.0% VAE and 4.0% CEO, yielding strong antibacterial activity against *E. coli* ATCC 25922 (15.50 mm inhibition) and *S. aureus* ATCC 25923 (16.71 mm inhibition), with a thickness of 0.15 mm, tensile strength of 0.32 Mpa, elongation of 0.32%, solubility of 4.89%, WVP of 8.82 g/m^2.h), and WVTR of 3.92 g/m.h. Morphological analysis of the edible film showed a relatively flat and smooth surface with minor cracks in some areas.

Keywords: antibacterial packaging, chitosan, cinnamon essential oil, edible film, velvet bean aqueous extract

INTRODUCTION

The current global warming crisis has encouraged extensive research into edible films. The use of edible film has emerged as an international trend and nowadays is important for products to compete in the global market. Edible films are an environmentally friendly packaging innovation to extend product shelf life (Arham et al., 2016). Edible film raw materials typically include proteins (polypeptides), carbohydrates (polysaccharides), and fats (lipids). These materials are thermoplastic, making them easy to mold into edible films with the added advantage of biodegradability (Widodo et al., 2019).

Most edible films are composed of polysaccharides. Polysaccharides that are commonly used for producing edible films include chitosan, pectin, alginate, and gelatin (Pirozzi et al., 2021). Chitosan is a multifunctional biopolymer for its 3 functional groups, namely amino acids and primary and secondary hydroxyl groups, all of which can improve the mechanical strength of bioplastic sheets (Rosida et al., 2018). Bioactive compounds from plants can be added to edible films to increase their biological activity, including their antibacterial properties (Pirozzi et al., 2021).

The effectiveness of the antibacterial properties of an edible film can be enhanced through the addition of bioactive components such as extracts or essential oils from several plant parts (rhizomes, leaves, stems, and seeds) (Pirozzi et al., 2021).Velvet bean is one plant with promising potential for increasing antibacterial activity. Its seeds have valuable medicinal

properties and have been investigated in various contexts, including antidiabetic, aphrodisiac, antineoplastic, antiepileptic, and antibacterial activities (Lampariello et al., 2012). This plant contains active compounds such as alkaloids, flavonoids, and tannins, which are known to have antibacterial potential. Several studies have shown that velvet bean seed extract can inhibit the growth of various types of pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli.*

Velvet bean is a tropical plant that is widely found in Indonesia, especially in DIY, Central Java, and East Java. However, despite its abundance, the use of velvet bean remains limited (Kusuma & Nur Oktaviani, 2022). Due to its complex processing requirements and lower popularity compared to other types of beans. Velvet bean is rarely applied commercially other than in animal feed and tempeh production (Ruma et al., 2021).

Another natural ingredient that can be added to edible films is essential oil. Indonesia is the center of biodiversity in the world for claiming 31,750 types of plants. This great figure has brought this country to have a great potential in producing essential oils oils (Yusdar, 2015). Currently, 150 types of essential oils are being traded internationally; 40 of them come from Indonesia (Jati, 2022). Essential oils have been used since ancient times as traditional medicine and food preservatives (Naeem et al., 2018). for their advantages, including antibacterial, antifungal (Yanti et al., 2020), and antioxidant properties (Yunilawati et al., 2021). Essential oils are widely used as edible film additives because they contain beneficial chemical components such as esters, aldehydes, ketones, terpenes, and phenolic compounds, which are beneficial for enhancing the bioactivity of edible films (Pirozzi et al., 2021).

Cinnamon essential oil is valuable for enhancing the antibacterial properties of edible films. Indonesia is the world's largest producer of cinnamon bark, contributing 46.0% of global production, followed by China (33.7%), Vietnam (10.1%), Sri Lanka (8.1%), and Madagascar 1.1% (Nurhayani & Rosmeli, 2019). Research by Ariani et al. (2024) revealed that cinnamon essential oil has two active antibacterial compounds, namely cinnamaldehyde and eugenol, which can damage bacterial cell proteins, thereby disrupting cell membranes or inactivating certain enzymes (Hikmatyar et al., 2017). Yunilawati et al. (2021) found that cinnamon essential oil has very strong antibacterial activity, with inhibition zones of 34.0 mm and 35.0 mm against *E. coli* and *S. aureus*, respectively.

Cinnamon essential oil (EO) demonstrates a variety of antimicrobial properties against foodborne pathogens when utilized in combination with other EOs. Specifically, the combination of cinnamon EO and oregano EO has been observed to exhibit a synergistic effect against *S. typhimurium* and an additive effect against *E. coli, Salmonella spp.,* and *L. monocytogenes.* A similar synergistic effect has been observed when cinnamon EO is combined with thyme EO, affecting *E. coli, Salmonella spp.,* and *S. typhimurium.* A synergistic effect has also been observed when cinnamon EO is combined with black pepper EO, affecting *L. monocytogenes* (Almeida et al., 2024; Alonso et al., 2024). The utilization of edible films composed of pectin and whey containing cinnamon essential oil demonstrated a significant level of antimicrobial activity against the associated food contaminants such as *E. coli, L. monocytogenes* and *S. aureus.* These findings suggest that this material could serve as a sustainable solution to conventional packaging materials specifically used in food processing (Sharma et al., 2017).

Zhou et al. (2021) have studied the effect of cinnamon essential oil addition on the thermal, physical, structural, and mechanical properties of cassava starch-based edible films. The results showed that edible film from cassava starch modified with cinnamon essential oil is very prospective to be developed. The results of research by Ariani et al. (2024) showed that the addition of 30% velvet bean ethanol extract and 3.0% cinnamon essential oil to chitosan-based edible film products can produce new edible films with antibacterial activity of *E. coli* ATCC 25922 of 19.36 mm (very strong) and *S. aureus* ATCC 25923 of 18.94 mm (very strong).

Recently, bioactive films with essential oils are able to provide more effective functional and sensory benefits to packaged products, can impart antimicrobial properties to the film, and also help prevent water evaporation from the food surface, thus slowing down the degradation process (Putra et al., 2017). The bioactive films offer technological advances in the field of packaging regarding an environmentally friendly approach to consumable food packaging. This approach is also an alternative to the growing demand for food packaging that implements antibacterial and sustainable protection by combining several natural products containing bioactive compounds with advanced materials for packaging (Apriani et al., 2019). From several references to the research results above, it is tried to be further developed regarding the production of chitosan-based edible films with the addition of velvet bean aqueous extract and cinnamon essential oil.

EXPERIMENTAL SECTION

Plant Determination

Velvet bean (*Mucuna pruriens* (L.) DC) plant was obtained from Wonogiri, Indonesia (110° 41'-111° 18' BT and 7° 32'-8° 15' LS) and determination had been carried out at the Laboratory of Plant Systematics, Faculty of Biology of Universitas Gadjah Mada with registration number 0701/S.Tb./X/2022. Cinnamon (*Cinnamomum verum* J.S. Presl) plant was obtained from Boyolali, Central Java, Indonesia (7° 7'-7° 36' N

and 110° 22'-110° 50' E) and determination was done at the Biology Laboratory, FMIPA Universitas Sebelas Maret with registration number 040/UN27.9.6.4/ Lab/2022.

Production of Velvet Bean Aqueous Extract (VAE)

Velvet bean seeds 750 g were weighed, then soaked and sorted for 3 x 24 hours. During soaking, the seed coat is peeled off. After 3 x 24 hours, 500 g of clean velvet bean seeds (washed and drained) were obtained. The beans were crushed with the addition of 250 mL distilled water as a solvent. Following this, the maceration process was carried out for approximately 6 hours (Ariani et al., 2016). The velvet bean pulp was then filtered to obtain the filtrate. The filtrate obtained was then evaporated until a thick consistency was (Almeida et al., 2024; Alonso et al., 2024). After that, the calculation of the yield of velvet bean water extract is carried out to determine how much bioactive compound content in the material can be extracted. The resulting velvet bean aqueous extract (VAE) was then tested for antibacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 (Ariani et al., 2022; Ariani, Hayus, Mulyani et al., 2024).

Production of Cinnamon Essential Oil (CEO)

Cinnamon bark was sorted, washed thoroughly and dried by air-drying. Then crushed using a pounder until it was in powder form. Cinnamon powder was weighed and obtained a weight of 3.47 kg. The cinnamon was put in a steam-water distillation apparatus, which was activated to extract the essential oil. In the distillation process are used aquades solvents because aquades can accelerate the dissolution of secondary metabolite compounds in cinnamon (Ariani, Rohmatun et al., 2024; Mulyanti et al., 2023). The resulting essential oil, initially mixed with water, was separated using a separating funnel. To remove any remaining water, anhydrous Na_2SO_4 was added (Sufyan & Destiarti, 2018). Once the essential oil was obtained, tests were conducted to measure its properties, including color, form, and aroma (Widodo et al., 2019). Finally, the cinnamon essential oil (CEO) was tested for antibacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923.

Identification of Cinnamon Essential Oil Components

The cinnamon essential oil (CEO) was analyzed for its chemical components using a Shimadzu QP 2010 GC-MS instrument. The GC-MS conditions were as follows: an Electron Impact (EI) operating system with an Rtx-MS column (30 m x 0.25 mm, 0.25 μm), injection and detector temperatures of 200°C, a column temperature of 60-330°C, and helium as the carrier gas. The results of mass spectra analysis were compared with existing data in the *Wiley7 Library* to identify the chemical components contained in the essential oil (Ariani, Mulyani, Hayus et al., 2023; Ariani, Mitsalina et al., 2024).

Optimization of Edible Film Formulation

Chitosan-based edible film (CE)

The edible film formulation referred to research conducted by Widodo et al. (2019) as shown in **Table 1.** The steps for preparing CE were as follows: 3.0 g of chitosan powder was dissolved in 100 mL of 1.0% CH_3COOH solution in a 250 mL beaker glass. The mixture was stirred with a magnetic stirrer until homogeneous, after which 1.0 mL of glycerol was added and stirred again for 30 minutes. Once the mixture was completely homogeneous, it was poured into a 10 x 10 cm glass mold and dried in an oven at 60° C for 6.0 hours. The resulting edible film was then tested for antibacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 (Ariani et al., 2022; Ariani, Hayus, Mulyani et al., 2024).

Chitosan-based edible film with velvet bean aqueous extract (CEVA)

The formulation of CEVA could be following **Table 2.** The steps for preparing ECVA were as follows: chitosan was dissolved in 100.0 mL of 1.0% CH_3COOH solution and stirred until homogeneous. Then, 1.0 mL glycerol was added and stirred again for 30 minutes. After the mixture was homogeneous, VAE was added at various concentrations (0, 10, 20, 30, 40, and 50) (% w/w). The edible film solution was then poured into a 10 x 10 cm glass mold and dried for 6.0 hours at 60° C. Finally, the edible film was tested for antibacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 to determine the best formulation (Ariani, Mulyani, Hayus et al., 2023; Santoni et al., 2024).

Chitosan-based edible film with velvet bean aqueous extract and cinnamon essential oil (CEVAC)

Based on the optimization of CEVA in terms of antibacterial activity, the best performing ECVA formulation was identified. This optimal CEVA was then further enhanced by adding cinnamon essential oil (CEO) in the following formulation as shown in **Table 3**.

Table 1. Chitosan-Based Edible Film (CE) Formulation (Widodo et al., 2019).

Material	Composition
Chitosan	3.0 (g)
CH_3COOH 1%	100.0 (mL)
Glycerol	1.0 (mL)

Table 2. The formulation of ECVA

Material	Formulation					
	CEVA 0	CEVA 10	CEVA 20	CEVA 30	CEVA 40	CEVA 50
Chitosan-based edible film (CE) (%)	100	90	80	70	60	50
VAE (%)	0	10	20	30	40	50
Total (%)	100	100	100	100	100	100

Description: CE = chitosan powder (3.0 g), CH_3COOH 1.0% (100 mL), and glycerol (1.0 mL)
CEVA= formulation of chitosan-based edible film with VAE

Table 3. Formulation of chitosan-based edible film with velvet bean aqueous extract and cinnamon essential oil (CEVAC)

Material	Sample Formulation					
	CEVAC 0	CEVAC 1	CEVAC 2	CEVAC 3	CEVAC 4	CEVAC 5
CEVA (%)	100	99	98	97	96	95
CEO (%)	0	1	2	3	4	5
Total (%)	100	100	100	100	100	100

Description: CEVA = Chitosan powder (3.0 g), CH_3COOH 1.0% (100 mL), glycerol (1.0 mL) and VAE
CEVAC = Formulation of chitosan-based edible film with VAE and CEO

Edible films made from chitosan with velvet bean aqueous extract and cinnamon essential oil (CEVAC) were tested for antibacterial activity to determine the best formulation.

Antibacterial Activity Test

The edible film's antibacterial activity was tested against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 using the well diffusion method. Chloramphenicol was used as a positive control for *E. coli* ATCC 25922, while vancomycin served as a positive control for *S. aureus* ATCC 25923 (Ariani et al., 2022; Ariani, Mulyani, Susilowati et al., 2023; Zainal et al., 2022).

Physical Properties Test

Furthermore, the optimal CEVAC, based on its antibacterial activity, was tested for physical properties, including thickness, tensile strength, elongation, solubility, WVP, and WVTR (Alamsjah et al., 2015; Dewi, 2019; Utama et al., 2018).

Morphological Analysis by the SEM Method

The CEVAC with the optimal antibacterial activities was analyzed morphologically using the SEM method (Bahar et al., 2023).

RESULTS AND DISCUSSION

Production of Velvet Bean Aqua Extract (VAE)

The velvet bean water extract obtained is brownish in colour and smells typical of velvet beans with a weight of 73.72 g and a yield of 14.7%. These results are in accordance with the results of velvet bean extraction in the research of Febriana & Kusuma (2022) which states that velvet bean extract obtained by maceration extraction method has thick characteristic, brown-black colour, and a distinctive smell of velvet bean with a yield of 14.01%. In this research, water was used as the extraction solvent to ensure that the extract added to the edible film product would be safe for consumption (Supriyanti et al., 2015). Additionally, water is a polar solvent, suitable for extracting polar compounds like L-Dopa in velvet beans. Research on L-DOPA in velvet beans has shown significant antibacterial potential, among other therapeutic properties. Velvet bean seed extracts have demonstrated antibacterial activity against various bacterial strains, including *E. coli*, indicating their potential as antibacterial agents (Jitpimai et al., 2023). Maceration was conducted at room temperature. This method was chosen to prevent degradation of the compounds (Indrawati et al., 2015; Wojdyło et al., 2021).

VAE's Antibacterial Activity Test Results

The following are the results of the velvet bean aqueous extract (VAE)'s antibacterial activity test. Based on **Table 4 and Figure 1** above, the antibacterial activity test of velvet bean aqueous extract against *E. coli* ATCC 25922 showed an inhibition zone of 33.18 (very strong), while that against *S. aureus* ATCC 25923 indicated an inhibition zone of 32.83 mm (very strong). The strong antibacterial activity of velvet bean aqueous extract is due to the presence of L-Dopa in velvet bean. The research results of Ariani et al. (2016) stated that steamed velvet bean extract contains 40.20% L-Dopa. Winarni & Dharmawan (2016) stated that koro benguk boiled for 1 hour followed by soaking for 2 days contained L-Dopa of 9781.55 ppm. The study of Habibah et al. (2022) stated that the content of L-Dopa compounds was 1,105 ppm / 20 g of velvet bean. The molecular formula of L-Dopa $C_9H_{11}NO_4$ is a non-protein amino metabolite with a molecular weight of 197.19 g/mol and a melting point of 270-284°C. L-Dopa is known to contain 2 phenol groups. Antibacterial activity can be ranked based on the groups in the chemical

compounds, namely phenols> aldehydes> ketones> alcohols (Yunilawati et al., 2021). The phenol group plays a role in inhibiting nucleic acid synthesis and energy metabolism of bacteria (Parwati et al., 2019).

Production of Cinnamon Essential Oil

The essential oils in this study were isolated using the steam-water distillation method because essential oils are volatile compounds. The steam-water distillation method can effectively isolate essential oils with low vapor pressure and boiling points while minimizing the risk of damage from hydrolysis and polymerization reactions that can occur due to heating (Akdağ & Öztürk, 2019; Rizki & Panjaitan, 2018).

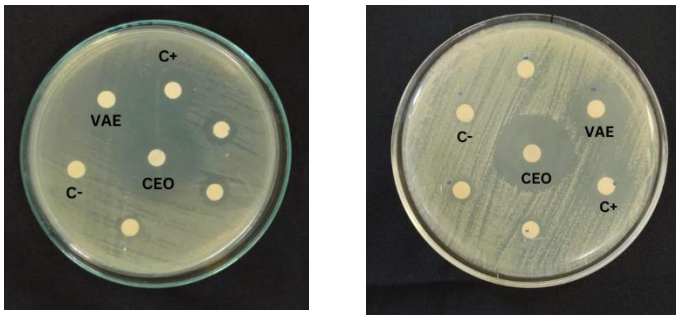
Identification of Cinnamon Essential Oil Components

The essential oil components were identified by the GC-MS method, with the resulting GC chromatograms presented in **Figure 2**. The identified chemical components of cinnamon essential oil are presented in **Table 6**. The compounds predicted to have antibacterial activity in CEO could are also listed in **Table 7**.

Table 4. Velvet bean aqua extract (VAE)'s antibacterial test results

Bacteria	Sample	Zone of Inhibition (mm)	Category
E. coli ATCC 25922	C- (distilled water)	0.00 ± 0.00	None
	C+ *(Chloramphenicol)*	21.79 ± 0.02	Strong
	VAE	33.18 ± 0.02	Very Strong
S. aureus ATCC 25923	C- (distilled water)	0.0 ± 0.00	None
	C+ *(Vancomycin)*	21.07 ± 0.03	Strong
	VAE	32.83 ± 0.02	Very Strong

Description: VAE = Velvet bean aqueous extract
C+ = Positive control
C- = Negative control



E. coli ATCC 25922 *S. aureus* ATCC 25923

Figure 1. Velvet bean aqua extract (VAE)'s and Cinnamon Essential Oil (CEO)'s antibacterial test results

Table 5. Cinnamon essential oil characterization results

Parameters	Results	Photo
Initial Weight (kg)	3.47	
Volume (mL)	10	
Color	Clear Yellow	
Form	Liquid	
Aroma	Typical Cinnamon	
Content (% v/w)	0.288	

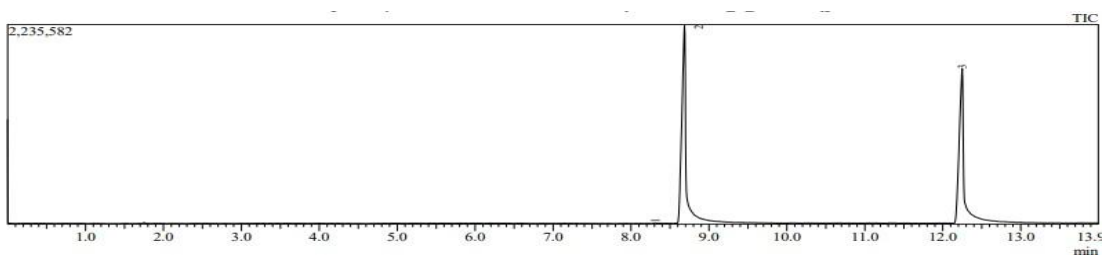


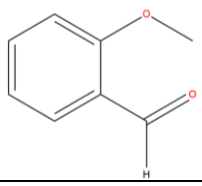
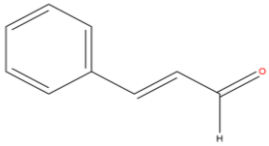
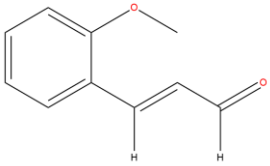
Figure 2. The GC chromatogram of cinnamon essential oil

Table 6. The cinnamon essential oil chemical component identification results

Retenti on Time (minute s)	% Area	Molecul ar Weight	MS Data Character: m/z (Relative Intensity)							Compound Name
8.312	0.09	136.14	40 (5)	51 (40)	65 (40)	77 (100)	92 (33)	104 (22)	118 (37)	*2-Methoxybenzaldehyde*
8.685	52.86	132.16	40 (2)	51 (45)	63 (9)	77 (50)	91 (3)	103 (58)	115 (2)	*Cinnamaldehyde*
12.249	47.06	162.18	40 (2)	51 (17)	65 (20)	77 (23)	91 (50)	103 (22)	119 (28)	*2-Methoxycinnamaldehyde*

Main Group of Compounds:
Aromatic Aldehyde Compounds (No. 1, 2, and 3) (%) = 100
Total Identified (%) = 100

Table 7. The antibacterial compound predicted in CEO

Compound Name	*2-Methoxybenzaldehyde*	*Cinnamaldehyde*	*2-Methoxycinnamaldehyde*
Content (%)	0.09	52.86	47.06
Chemical Structure			

The identifiable chemical components in cinnamon essential oil were 2-methoxybenzaldehyde (0.09%), cinnamaldehyde (52.86%), and 2-methoxycinnamaldehyde (47.06%). Only two chemical compounds are involved in essential oil, namely cinnamaldehyde and 2-methoxycinnamaldehyde. The content of methoxybenzaldehyde is almost insignificant. These three compounds belong to the class of aromatic aldehydes, known for their antibacterial properties. Essential oils containing aldehyde groups are the main components. Antibacterial activity can be ranked based on the compound's functional group, as follows: phenol> aldehyde> ketone> alcohol> ester> hydrocarbon (Yunilawati et al., 2021). Cinnamon, according to Djarot & Ambarwati (2019), contains mainly cinnamaldehyde (60.72%), eugenol (17.62%), and coumarin (13.39%). (Rizki & Panjaitan, 2018) also identified cinnamaldehyde (60.72%) and cinnamyl acetate (24.88%) as the primary components in cinnamon. The GC-MS results revealed differences in chemical composition compared to previous studies. This may be attributed to factors such as differences in plants, growing environment, density of raw materials selected, and different isolation methods, all of which can influence essential oil composition (Mehalaine & Chenchouni, 2020; Sufyan & Destiarti, 2018). Different parts of the cinnamon plant contain varying proportions of hydrocarbons. The main constituents in cinnamon include cinnamaldehyde in the bark, eugenol in the leaves, and champor in the roots (Purwakanthi & Rahman, 2021). Bioactive phytochemicals that play an important role in cinnamon antibacterial include cinnamaldehyde and eugenol (El Atki et al., 2019). Cinnamaldehyde is an electronegative molecule that can interfere with nitrogen-containing cellular biological processes, such as those involving proteins and nucleic acids (Purwakanthi & Rahman, 2021).

CEO Antibacterial Activity Test Results

The results of the cinnamon essential oil antibacterial activity test are presented in **Table 8**. Based on **Figure 1** and **Table 8**, the antibacterial activity of cinnamon essential oil (CEO) against *E. coli* ATCC 25922 showed an inhibition of 21.79 mm (very strong), while the inhibition zone for the *S. aureus* ATCC 25923 was 47.95 mm (very strong). This is based on the inhibition zone category which states that if the inhibition zone is greater than 20 mm then the inhibition zone is categorized as very strong (Pangouw et al., 2020).

The bioactive phytochemicals that play an important role as antibacterial in cinnamon include cinnamaldehyde and eugenol (Hikmatyar et al., 2017). The antimicrobial mechanism of cinnamon essential oil against *E. coli* and *S. aureus* is caused by aldehyde compounds in its main components. These compounds cause membrane lysis, electrolyte leakage due to impaired cell permeability, and depolarization of cell membranes, which cause irregular cell metabolic activity and bacterial death (Yunilawati et al., 2021).

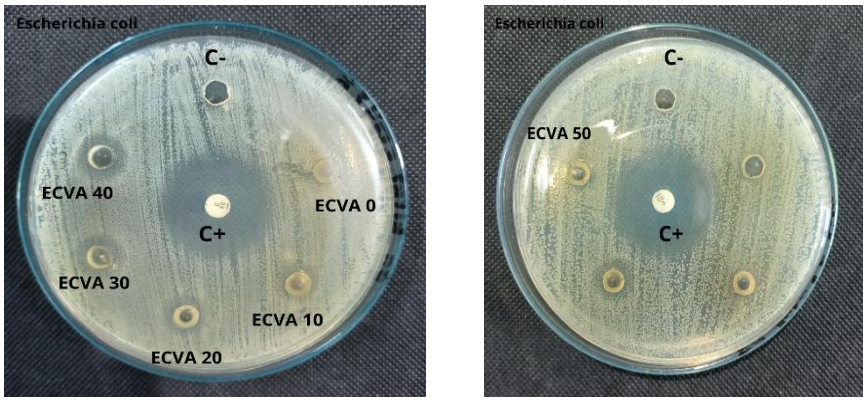
CEVA Antibacterial Activity Test Results

The results of the antibacterial activity test against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 can be seen in **Figure 3** and **Table 9** below. In the antibacterial activity test against *E. coli* ATCC 25922, the highest inhibition was observed at a 30% VEA, with an inhibition zone of 12.06 mm, after which the inhibition decreased. In the *S. aureus* ATCC 25923 test, the highest value was obtained at a 30% VAE concentration, with an inhibition zone of 7.70 mm. The differences in the inhibition values could be due to variations in the concentration of VAE and the active compounds present in the edible film. Based on these results, it could be concluded that the addition of VAE can enhance the antibacterial activity of edible films.

Table 8. The results of the CEO antibacterial activity test

Bacteria	Name	Zone of inhibition (mm)	Category
E. coli	C- (distilled water)	0.00 ± 0.00	None
ATCC 25922	C+ *(Chloramphenicol)*	21.79 ± 0.02	Very Strong
	CEO	46.28 ± 0.03	Very Strong
S. aureus	C- (distilled water)	0.00 ± 0.00	None
ATCC 25923	C+ *(Vancomycin)*	21.07 ± 0.03	Very Strong
	CEO	47.95 ± 0.02	Very Strong

Description:
CEO = Cinnamon essential oil
C+ = Positive control
C- = Negative control



E. coli ATCC 25922

S. aureus ATCC 25923

Description

CE	:	Chitosan-based edible film			
CEVA 0	:	CE + 0% VAE	CEVA 40	:	CE + 40% VAE
CEVA 10	:	CE + 10% VAE	CEVA 50	:	CE + 50% VAE
CEVA 20	:	CE + 20% VAE	C+	:	Positive control
CEVA 30	:	CE + 30% VAE	C-	:	Negative control

Figure 3. Results of antibacterial activity test of chitosan-based edible film with velvet bean aqueous extract (CEVA)

Table 9. The results of CEVA antibacterial activity test

Bacteria	Name	Zone of Inhibition (mm)	Category
E. coli ATCC 25922	C- (distilled water)	0,00 ± 0,00	None
	C+ *(Chloramphenicol)*	26,58 ± 0,03	Very Strong
	ECVA 0	9.53^{a} ± 0.06	Medium
	ECVA 10	10.46^{b} ± 0.02	Medium
	ECVA 20	10.66^{c} ± 0.04	Medium
	ECVA 30	12.06^{f} ± 0.05	Strong
	ECVA 40	11.93^{e} ± 0.02	Strong
	ECVA 50	10.85^{d} ± 0.02	Medium
S. aureus ATCC 25923	C- (distilled water)	0,00 ± 0,00	None
	C+ *(Vancomycin)*	25,78 ± 0,02	Very Strong
	ECVA 0	2.24^{a} ± 0.03	Weak
	ECVA 10	7.22^{d} ± 0.02	Medium
	ECVA 20	7.07^{b} ± 0.04	Medium
	ECVA 30	7.70^{f} ± 0.02	Medium
	ECVA 40	7.28^{e} ± 0.03	Medium
	ECVA 50	7.17^{c} ± 0.07	Medium

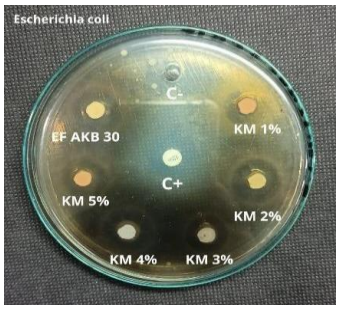
Description

CEVA	:	Chitosan-based edible film with VAE			
CEVA 0	:	CE + 0% VAE	CEVA 40	:	CE + 40% VAE
CEVA 10	:	CE + 10% VAE	CEVA 50	:	CE + 50% VAE
CEVA 20	:	CE + 20% VAE	C+	:	Positive control
CEVA 30	:	CE + 30% VAE	C-	:	Negative control

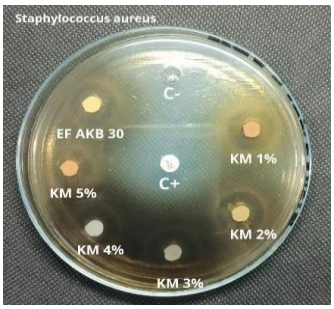
Results of CEVAC Antibacterial Activity Test of

The results of CEVAC's bacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 are shown in **Figure 4**. The results of CEVAC's antibacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 are shown in **Table 10**.

In the antibacterial activity test against *E. coli* ATCC 25922, the inhibition increased with the addition of CEO until reaching a concentration of 4%, where an inhibition zone of 15.50 mm was observed. The inhibition then fluctuated at higher concentrations. In the *S. aureus* ATCC 25923 test, the inhibition was greater, peaking at a 4% CEO concentration with an inhibition zone of 16.71 mm, after which it fluctuated. The difference in the inhibition values could be due to differences variations in the concentration of CEO and the active compounds in the essential oil.



(a) *E. coli* ATCC 25922



(b) *S. aureus* ATCC 25923

Description:

CEVAC	:	Chitosan-based edible film b with VAE and CEO
CEVAC 0	:	CE+30% VAE+0% CEO
CEVAC 1	:	CE+30% VAE+1% CEO
CEVAC 2	:	CE+30% VAE+2% CEO
CEVAC 3	:	CE+30% VAE+3% CEO
CEVAC 4	:	CE+30% VAE+4% CEO
CEVAC 5	:	CE+30% VAE+5% CEO
C+	:	Positive control
C-	:	Negative control

Figure 4 The results of CEVAC antibacterial activity test

Table 10. The results of CEVA antibacterial activity test

Bacteria	Name	Zone of Inhibition (mm)	Category
Escherichia coli ATCC 25922	C- (distilled water)	0.00 ± 0.00	None
	C+ *(Chloramphenicol)*	25.87 ± 0.07	Very Strong
	CEVAC 0	9.97^{a} ± 0.03	Strong
	CEVAC 1	13.33^{c} ± 0.03	Strong
	CEVAC 2	14.08^{e} ± 0.04	Strong
	CEVAC 3	12.02^{b} ± 0.06	Strong
	CEVAC 4	15.50^{f} ± 0.4	Strong
	CEVAC 5	13.98^{d} ± 0.03	Strong
Staphylococcus aureus ATCC 25923	C- (distilled water)	0,00 ± 0,00	None
	C+ *(Vancomycin)*	29.03 ± 0,04	Very Strong
	CEVAC 0	13.46^{a} ± 0.02	Strong
	CEVAC 1	14.07^{b} ± 0.03	Strong
	CEVAC 2	14.31^{c} ± 0.02	Strong
	CEVAC 3	15.03^{d} ± 0.02	Strong
	CEVAC 4	16.71^{f} ± 0.02	Strong
	CEVAC 5	15.50^{e} ± 0.02	Strong

Description:
CEVAC : Chitosan-based edible film b with VAE and CEO
CEVAC 0 : CE+30% VAE+0% CEO
CEVAC 1 : CE+30% VAE+1% CEO
CEVAC 2 : CE+30% VAE+2% CEO
CEVAC 3 : CE+30% VAE+3% CEO
CEVAC 4 : CE+30% VAE+4% CEO
CEVAC 5 : CE+30% VAE+5% CEO
C+ : Positive control
C- : Negative control

Antibacterial bioactive phytochemicals that play an important role in cinnamon include cinnamaldehyde and eugenol (Hikmatyar et al., 2017). In films incorporating essential oils, oil droplets are physically or chemically trapped in the polymer matrix. The presence of chemical bonds between essential oils and polymer functional groups leads to strong interactions that reduce the migration rate of active components, which allows a slower and gradual diffusion of essential oils to the environment in food packaging. In some applications, slow release of active compounds from the packaging system is required to improve product durability during storage (Yunilawati et al., 2021). Based on the results of the antibacterial test above, it could be concluded that the addition of cinnamon essential oil can increase antibacterial activities and reach a maximum at the addition of 4% cinnamon essential oil.

Results of Characterization of the Physical Properties of CEVAC with the Most Optimal Antibacterial Activity

Physical quality testing was conducted to determine the quality of CEVAC. The parameters measured in this study include thickness, tensile strength, elongation, solubility, WVP, and WVTR. The following are the results of the analysis of the physical properties of CEVAC with the highest antibacterial activity.

Thickness

Thickness is an important characteristic in determining the feasibility of edible films as food packaging, as it greatly affects the physical and mechanical properties of edible films (Rusli et al., 2017). The average thickness of CEVAC was 0.15 mm. The addition of essential oil does not affect the total solids in the edible film suspension, thus not increasing the thickness of the edible film (Sulaiman et al., 2023). Thickness, in turn, affects other properties of the edible film, such as tensile strength, percent elongation, and gas permeability (Syarifuddin & Yunianta, 2015). Factors that affect the thickness of edible films include the amount of soluble solids and the area of the plate used (Agustini et al., 2021; Putra et al., 2017).

Tensile strength

Tensile strength indicates the maximum force required to break the edible film (Syarifuddin & Yunianta, 2015). The average tensile strength of CEVAC was 0.32 Mpa, meeting the minimum requirements of the Japanesse International Standard (JIS) (1975) of 0.3 Mpa. However, the value obtained was close to the minimum requirement. Lower tensile strength values indicate that the edible film is more brittle and easily broken, while higher t values indicate greater

durability (Afifah et al., 2018; Hayati et al., 2020). In this research, the CEVAC had low tensile strength, likely due to challenges in homogenizing the mixture, resulting in a more brittle film.

Elongation

Elongation depicts the ability of the film to stretch, which depends on the type of material used in the edible film and affect the cohesion properties of the film's structure. The average elongation of CEVAC was 0.32%. The elongation of an edible film is influenced by its constituent, including hydrophilic materials that cause the formation of free spaces and increase molecular mobility to form hydrogen bonds. The flexibility of edible films can be influenced by the polarity of the compounds that form them. The flexibility properties of edible films can cause water-polymer bonds to form, reducing polymer-polymer interactions and increasing flexibility. In this edible film, glycerol can reduce internal hydrogen bonds by creating more space between molecules, thereby reducing rigidity and increasing flexibility (Li et al., 2024; Syarifuddin & Yunianta, 2015).

Solubility

Solubility refers to the percentage of film solubility in distilled water over a specific period. A higher percentage of solubility indicates the easier the edible film to consume. The average solubility of CEVAC was 4.89%. The solubility of this edible film is influenced by the glycerol component, which is hydrophilic and thus dissolves in water (Syarifuddin & Yunianta, 2015). The data in the table shows that the higher the concentration of essential oil added, the lower of the solubility value. This is because the hydrophobic nature of the essential oils can reduce the hydroxyl groups in the edible film. Hydroxyl groups that are easily soluble in water are replaced by the hydrophobic groups from the essential oils. Furthermore, other materials that are hydrophilic in edible film formulation also enhance the solubility of edible film (Choi et al., 2022; Putra et al., 2017).

WVP

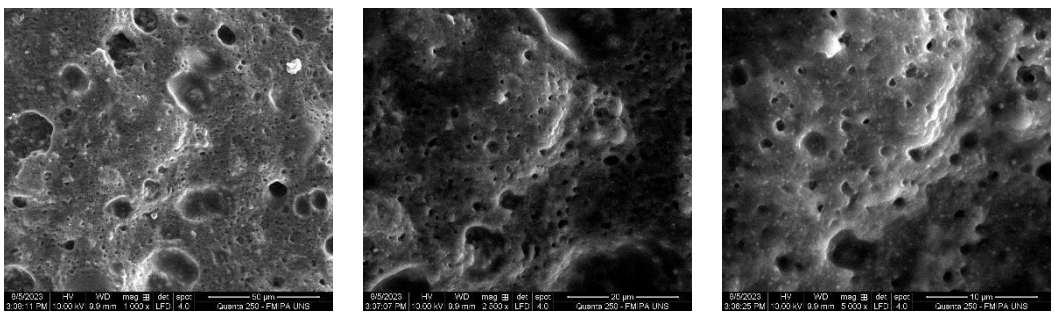
Water Vapor Permeability (WVP) means the rate at which water vapor passes through the film per unit time, divided by the film area (Adjouman et al., 2017; Sulaiman et al., 2023). The average WVP of CEVAC was 8.82. The concentration of essential oil used does not have a significant difference, which is why the WVP value does not remain relatively consistent across the various formulations.

WVTR

Water Vapor Transmission Rate (WVTR) or commonly known as water vapor transmission rate is a parameter to determine the ability of edible film to inhibit the transmission of water vapor from the coated material (Rachmawati et al., 2023). The average WVTR of CEVAC was 3.92. This is different from the research by Putra *et al.* (2017), where the addition of kaffir lime leaf essential oil concentration lowered the WVTR of the edible film. However, in this study, no significant difference in WVTR was observed with varying concentrations of cinnamon essential oil. Edible films with a more compact structure and network can increase their ability to retain water vapor. According to Japanese Industry Standards, the lower the WVTR value, the better quality of edible film in terms of protecting the product, slowing down oxidation, and maintaining product integrity (Rachmawati et al., 2023).

Table 11. The results of the physical properties characterization of CEVAC with the highest antibacterial activity

Repetition	Thickness (mm)	Tensile strength (Mpa)	Elongation (%)	Solubility (%)	WVTR (g/m^2.day)	WVP (g/m.day)
1	0.150	0.330	0.330	4.890	3.920	8.823
2	0.140	0.320	0.320	4.880	3.930	8.820
3	0.160	0.310	0.310	4.900	3.910	8.823
Average	0.150	0.320	0.320	4.890	3.920	8.822
St Dev	0.010	0.010	0.010	0.010	0.010	0.002



(a) (b) (c)

Figure 5. The results of morphological analysis of CEVAC by the SEM method with magnifications of: (a) 1000x, (b) 2500x, and (c) 5000x

Morphological Analysis of CEVAC with the Most Optimal Antibacterial Activity Using the SEM Method

The results of morphological analysis of CEVAC with the most optimal antibacterial activity using the SEM method at 3 magnifications (1000x, 2500x, and 5000x) are shown in **Figure 5**.

The morphology of the edible film appeared flat and quite smooth, though slight cracks were present in some parts. This is believed to be due to insufficient mixing of the constituent materials, which made the edible film more fragile (Sulaiman et al., 2023). The irregularities found on the edible film surface were caused by the presence of macromolecules within the film (Asria, 2016; Supeni et al., 2015).

CONCLUSIONS

The conclusions of this study were: (a) CEO contained cinnamaldehyde (52.86%) and 2-methoxycinnamaldehyde (47.06%); (b) CEO had antibacterial activity against *E. coli* ATCC 25922 with an inhibition zone of 46.28 mm (very strong) and against *S. aureus* ATCC 25923 with an inhibition zone of 47.95 mm (very strong); (c) The chitosan-based edible film with the addition of 30.0% VAE and 4.0% CEO produced the most optimal formulation with antibacterial activity values of 15.50 mm (strong) against *E. coli* ATCC 25922 and 16.71 mm (strong) against *S. aureus* ATCC 25923, a thickness of 0.15 mm, tensile strength of 0.32 Mpa, elongation of 0.32%, solubility of 4.89%, WVP of 8.82 g/m^2.h, and WVTR of 3.92 g/m.h. The morphology of the edible film appeared flat and quite smooth, but there were minor cracks in some parts.

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