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Synthesis of Smart Packaging based on Chitosan-PVA/Binahong Extract as an Antibacterial Plastic

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Abstract. Chitosan-PVA composite with the addition of binahong extract is a bioplastic that is being developed aims to make eco-friendly product and contain antibacterial agents. This bioplastic is useful as packaging to maintain the freshness quality of food. The purpose of this research is to study and develop smart packaging with the addition of binahong leaf extract as an antibacterial agent. The manufacture of plastic in this study was carried out by varying the addition of binahong leaf extract ranging from 60-100%. The characteristics of the chitosan-PVA plastic were tested through FTIR, water uptake, and antibacterial tests. Chitosan-PVA plastic with the addition of 100% concentration of binahong extract had a good bacterial inhibition of 12 mm. The resulting chitosan-PVA plastic with binahong extract showed the presence of chemical bonds between the chitosan, PVA and flavonoid groups. The bioplastics produced have quite a large water uptake compared to non-biodegradable commercial packaging, reaching 491.52% with the addition of 90% binahong extract with the best antibacterial capabilities in the packaging with the addition of 100% binahong extract.

Introduction

To extend the shelf life of foodstuffs consumer preferences are shifting from the use of chemical preservatives to natural preservatives. The development of using biomaterial food packaging as a substitute for synthetic chemical preservatives has become a concern. The main problem is because eventually accumulation of plastic need thousands of years to be decomposed by the nature. This has become concerned both in social and environmental issues. Because of this reason, in recent years, significant studies have dedicated to exploring in order to develop packaging materials that not only act as antimicrobials to extend shelf life of food but also as biodegradable packaging. Chitosan is a noteworthy example of a natural polymer that offers protection against contaminant and microorganism in meals. Its versatility in modification and compatibility with other materials enables the formation of film coatings. Not only because of its ability to be degraded in nature, this polymer is one of the antimicrobial polymers. The presence of antimicrobial agents on packaging plays a crucial role in extending the shelf life of food by inhibiting the growth of microorganisms on the food surface upon contact with the film (Moreira et al., 2011; Van Den Broek et al., 2015). Chitosan has been recognized for its antimicrobial properties against a wide range of foodborne filamentous fungi, bacteria, and yeast (Helander et al., 2001), (No et al., 2007).

Effective packaging plays a vital factor to safeguarding food from damage caused by microorganisms, chemicals and the environment. Environmental factors such as light, temperature and water accelerate the deterioration of food quality. In recent decades, many researches have been extensive focusing on plastic film packaging from natural materials like chitosan. Cost effectiveness and eco-friendly properties have contributed to its prominence in the packaging industry sector.

Studies constructed by (Suyatma et al., 2004) demonstrate the enhanced aerodynamics properties of chitosan when combined with commercial polymer poly (lactic acid) (PLA). Likewise, (Kamel et al., 2004)

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discovered that the incorporation of 0.3% chitosan with 5% polyvinyl alcohol (PVA) provided optimum physical properties on the film. (Apriyanto Joko, 2007) Apriyanto (2007) [7] and Piluharto (2017) (Piluharto et al., 2017) conducted similar investigations. Focusing in the development of chitosan blending with PVA, Apriyanto revealed that chitosan: PVA (1:4) resulted notable enhancement in tensile strength, elongation percentage, and film thickness. Consistent with Apriyanto, in Piluharto's study (2017) (Apriyanto Joko, 2007; Piluharto et al., 2017) observed the same result in hydrogel chitosan/PVA (1:3).

In this study, a novel approach was employed to create smart packaging by combining chitosan–PVA with binahong leaf extract. The combination of these three components has not been explored. Chitosan was chosen for its versatility in modification and ability to be blended with other materials. Furthermore, chitosan is a natural preservative known for its ability to inhibit microbial growth (Siti Nur Holipah et al., 2010). However, chitosan is insoluble in water therefore, it is necessary to be blended with elastic biomaterial, water-soluble, and biodegradable, such as polyvinyl alcohol (PVA). On the other side, binahong extract has long been recognized for its traditional medicinal properties, including wound healing, and contains antioxidant agents (Selawa et al., 2013). It also acts as antifungal, antibacterial, anti-inflammatory, and antiviral properties due to the presence of secondary metabolites, such as alkaloids and steroids (Kurniawan & Aryana, 2015). A phytochemical screening conducted by (Garmana et al., 2014) Garman et al. confirmed the presence of flavonoids, saponins, and steroid/triterpenoid compounds in binahong leaf extract, which contributes to its antibacterial, antifungal effects, and anti-inflammatory.

Blending multiple polymers offers the opportunity to synthesize new materials with desired properties. The purpose of mixing is to achieve compatibility between the polymers for a specific application. For instance, chitosan, known for its rigidity and insolubility in water, can be blended with other polymers that possess elasticity and solubility in water when used as plastic. In this research, chitosan is utilized for food packaging, aiming to obtain a polymer with low water permeability to prevent food spoilage.

As a natural polymer, chitosan needs to be combined with a natural-based material that enables it to biodegrade in the environment. To enhance the mechanical properties of chitosan, synthetic biodegradable plasticizers are added (Thakhiew et al., 2015). One commonly used plasticizer in biodegradable polymers is polyvinyl alcohol (PVA).

PVA's water-soluble nature makes it eco-friendly and convenient for production. Moreover, its elasticity making it an ideal candidate for composites with chitosan. The impact of PVA on chitosan has been documented in previous research (Srinivasa et al., 2003), where chitosan-polyvinyl alcohol films exhibited variations in tensile strength (32-47 MPa) and elongation at break percentages (26.8-70.55%). The presence of polyvinyl alcohol increases the percentage of polymer elongation and the breaking value of the chitosan-PVA composite (Bonilla et al., 2014; Srinivasa et al., 2003).

Based on the considerations above, it becomes imperative to advance the development of current food packaging approaches. This involves not only utilizing chitosan and PVA as biofilms and antibacterial plastics but also additional binahong extract is a novel aspect that has not been explored by previous researchers.

Experimental

Material and Methods

The tools used in this experiment are glassware, hot plates, molds (glass/plastic plates), incubators, autoclaves, ovens, laminar air flow, FTIR, cupboards. cooler, rotary evaporator.

The materials used in this study were chitosan (pharmaceutical grade), polyvinyl alcohol (Merck), methyl orange (merck), binahong leaves, acetic acid, sodium tripolyphosphate, (technic), alcohol 96%, distilled water, tween 80 (merck), HCl (merck), magnesium and nutrient agar.

Procedures

Manufacturing of Nanochitosan

Preparation of Binahong Leaf Extract. Preparation of extract refers to (Sari et al., 2021). Binahong leaf extract is made from 500 grams of binahong leaves soaked in 1000 ml of 96% alcohol for 12 - 48 hours. Next, it is filtered to separate the filtrate and residue. The filtrate then was evaporated using a rotary evaporator at a speed of 65 rpm and a temperature of 60^{o}C. The binahong leaf extract obtained was dark green.

Phytochemical Screening

Phytochemical screening was carried out on the flavonoid content test refers to (Primadevi & Nafi'ah, 2020). 1 gram of extract was dissolved in 10 ml of distilled water, then 1 ml of the solution was taken and put into a test tube. The extract solution in the test tube was added with concentrated HCl and Mg powder (flavonoid test).

Preparation of Chitosan

The preparation of the chitosan solution refers to (Primadevi & Nafi'ah, 2020) with some modifications. 0.2 gram of chitosan was homogenized with a magnetic stirrer for 30 minutes in 100mL of 1% glacial acetic acid. Once dissolved, 0.1% STPP and 0.1% citric acid (5:1) were added to it, followed by 50 microliters of 0.1% tween 80 as a homogenizer. Furthermore, the homogenization process was continued for up to 2 hours using a magnetic stirrer. Chitosan-STPP solution added 1:1000 methyl orange. The final stage is the addition of binahong extract with various concentrations of 60%, 70%, 80%, 90% and 100%.

Fabrication of Chitosan-PVA Films

Preparation of a homogeneous chitosan-STPP solution by dissolving 0.3 gram of PVA in 10 mL of distilled water, adding 10 ml of nanochitosan solution (1:1), and stirring using a magnetic stirrer at a speed of 300 rpm and heated at 80°C for 30 minutes until film chitosan-PVA was formed.

Chitosan-PVA Film Characterization

Chitosan-PVA Film Functional Group Analysis. To determine the functional groups formed in chitosan-PVA, the FTIR spectra of the prepared films were carried out with an FTIR spectrophotometer. The spectrum was recorded in the range from 400 to 4000 cm^{-1}

Water Uptake. It is based on testing (Pirsa et al., 2018). The water resistance was tested by weighing the initial weight of the sample (wo), and then placing it in a container containing distilled water for 10 seconds. The sample was removed from distilled water and the water on the plastic surface was removed with tissue, then weighed as (w).

Furthermore, the water absorbed by the sample is calculated through the equation (1) :

$$\text{water uptake } (\%) = \frac{(w - w_o)}{w_o} \text{ x } 100\ \% \qquad (1)$$

W_o is the weight of the wet film and W is the weight of the dry film.

Antibacterial Test. Antibacterial test was performed using coliform *E. coli* bacteria. Chitosan-PVA films with concentrations of binahong 60%, 70%, 80%, 90%, and 100% were prepared into 1 x 1 cm sizes. The film placed on NA media which already contained *e-coli* bacteria then incubated for 1 day at room temperature.

Result and Discussion

Synthesis of chitosan powder

The ionic gelation with bottom-up principle method is a widely utilized technique for synthesizing nanochitosan. It is favored in research due to its simplicity and cost-effectiveness. The fundamental principle of this method revolves around the electrostatic interaction between the positively charged amino groups of chitosan and a negatively charged polyanion, such as sodium tripolyphosphate (STPP). To begin, chitosan is dissolved in 1% acetic acid, resulting in the generation of cations on the amine groups of chitosan.

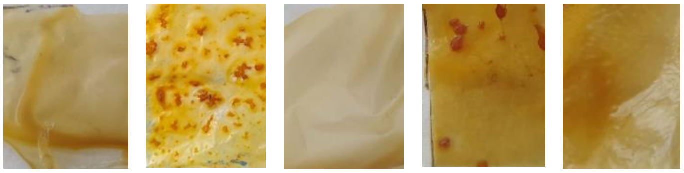
Subsequently, STPP is introduced as a crosslinking agent. STPP acts as an effective crosslinking agent due to its multivalent properties facilitating easy modification and blending with other composites. The convenient modification process helps prevent potential damage to the material to be encapsulated within the chitosan nanoparticles (Fan et al., 2012). Furthermore, STPP is considered harmless and food-grade packaging. The complex interaction between cations from chitosan and anions from STPP leads to the formation of chitosan polymer spheres, resembling a ball-like structure. As a result, nanoparticles are spontaneously formed through mechanical stirring at room temperature.

The size of the nanopolymer can be adjusted by finding an appropriate ratio between polymer and emulgator, as suggested by Irianto (2011). In the study conducted by Primadevi & Nafi'ah (2020), the impact of the crosslinking agent on nanochitosan was observed, resulting in a nanochitosan-TPP particle size of 5860.7 nm. To achieve a uniform composite with a smaller polymer size, the addition of tween 80 is necessary. Tween 80 not only acts as a surfactant but also enhances solubility between substances.

By utilizing the ionic gelation cross-linking method for nanoparticle preparation, smaller-sized chitosan can be obtained, as highlighted by Tiyaboonchai (2003). Nanoparticles are colloidal particles that solidify and typically have diameters ranging from 1-1000 nm. The nanoscale size enhances the antibacterial properties of the plastic due to the increasing adsorption of chitosan into bacterial cells (Qi et al., 2004). As an antibacterial bioplastic, chitosan film is supported by binahong extract that serves as an antibacterial substance in various concentrations, furthermore, it enhances its antibacterial effectiveness. Furthermore, the size of the powder that is produced should be tested by SEM or nanoparticle analyzer to know its exact particle size.

Preparing of chitosan-PVA films

In this study for the production of PVA films, the polymer dissolved in 80°C aquadest as suggested by (Sheftel VO, 2000). Once the PVA dissolved, it was mixed with nanochitosan using a stirrer to ensure a homogeneous composite. The film solution was then poured into plastic molds and dried in an oven at 50°C for 24 hours.



(a) Binahong 60% (b) Binahong 70% (c) Binahong 80% (d) Binahong 90% (e) Binahong 100%

Figure 1. Various of additional binahong in Ch-PVA film.

The films obtained from the five variations of binahong extract exhibit different visual appearances as served in Figure 1. Chitosan films (b) and (d) have rough surfaces due to a shorter stirring process and lower heating temperature. Conversely, the surfaces of the other chitosan films appear smoother. However, all films exhibit similar flexibility, easy to remove from the plate or mold, and resistance to tearing.

PVA functions as a plasticizer, providing strength to the polymer. This property enables the film to deform under pressure in the other word it allowing the polymer chains to move without damaging the film.

In various literatures, the use of chitosan films has been found to extend the shelf life of fruits. This is attributed to the semi-permeable nature of the film, which regulates gas exchange, slows down the respiration process, and controls the release of ethylene gas, a ripening agent in fruits. Furthermore, chitosan exhibits antifungal properties that can stimulate host defense responses, including the accumulation of antifungal compounds and phytoalexins (H. Li & Yu, 2001).

Chitosan-PVA Film Characterization

Functional group analysis of chitosan-PVA films. FTIR analysis allows the identification of unique functional groups present in chitosan, PVA, and flavonoids by analyzing the peaks at specific wave numbers resulting from their interaction with infrared light as served in Figure 2. Chitosan is believed to undergo physical and chemical chelation with both PVA and flavonoids in binahong extract. In this study, the peak in certain wave number obtained from the experiment will be compared with the chitosan and PVA standards resulted in tabel 2 (Dompeipen, 2017).

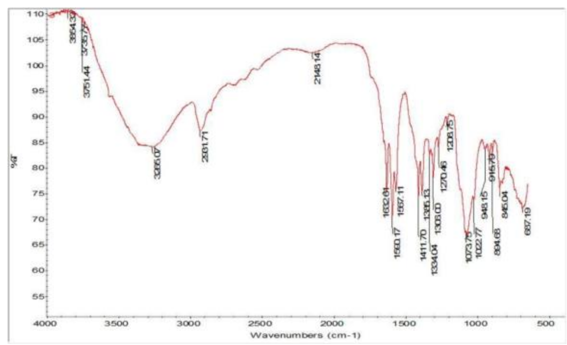


Figure 2. FTIR spectrums of Ch-PVA with 80% extract binahong.

Table 1. FTIR Spectrum of Chitosan.

Wave Number (cm^{-1})		Functional Group
Ch-PVA 80% of binahong (cm^{-1})	**Chitosan Standart (cm^{-1})**	
3265	3377,3302 [1]	O-H
2931,71	2922	C-H alifatik
-	2361,41	C-H aromatik
2148	-	-
1632,61	1666,30; 1660 [3]	C=H aromatik C=O amida I (pada kitosan [3]) (pada 1650 [4] cm^{-1} merupakan C-N pada $NHCOCH_3$),
1590, 1567	1587,94	Amida II (N-H bending
1411,1385	1417 [2]	CH_3 streching
1334	1324 [2]	deformasi CH_3 [2], C-N [2] (amida III)
1306	1318 [2]	
1270,46	1290 [3]	C-N [3] (amida II)

Wave Number (cm^{-1})		Functional Group
Ch-PVA 80% of binahong (cm^{-1})	**Chitosan Standart (cm^{-1})**	
-	1259	C-O
-	1154	C-O
1073	1077	O-H dan NH sekuder, C-O-C bending
915	-	P=O
894	897	β-1,4 glikosidik
845	-	C-H aromatik [5]
687	-	P-O [6]

Sources: [1] (Dompeipen, 2017) [2] (More et al., 2021) [3] [26] [4] (Silverstein, RM et al., 1989) [5] (Yadnya Putra et al., 2020a) [6] (Ferreira Tomaz et al., 2018)

Table 1 shows several absorption peaks that correspond to characteristic functional groups in chitosan-PVA (Ch-PVA). there were absorption, absorption peaks were observed at 3265.07 cm^{-1} meanwhile from Dompeipen (2017) the wave number at 3302.20 cm^{-1}, indicating the presence of bending vibrations of the O-H and NH groups. Literature suggests that the peak at 1650 cm^{-1} signifies the presence of the C=O group in the (-$NHCOCH_3$) bond. Another peak at 1632.61 cm^{-1} indicates the presence of the C-N group in chitosan-PVA, distinguishing it from the OH and NH groups (Jiao et al., 2017). Interestingly, the peak at 1632.61 cm^{-1} can also indicate the presence of aliphatic C-H groups derived from flavonoid compounds, which are secondary metabolites found in the binahong extract.

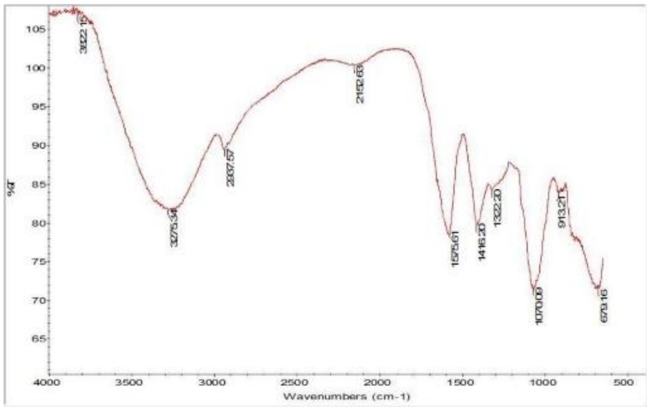
Comparing the findings with those (Veronita & Wijayati, 2017), the presence of the OH spectrum indicates alcohol compounds that are observed in the wave number region of 3381 cm^{-1}. This peak overlaps with the secondary amine groups in chitosan, as identified in the current study. According to (Putri et al., 2018) the flavonoid compounds form -NH_3^+-O interactions with chitosan, leading to ionic interactions known as ionic gelation. This technique promotes the formation of smaller particle sizes, including nano-sized particles.

According to the research conducted by (More et al., 2021), the C=O group appears at an absorption peak of 1662 cm^{-1}, indicating the vibration resulting from the bond between chitosan and vinyl acetate in PVA. Another typical vibration in PVA is the C-H bending at 845 cm^{-1}, which is not present in pure chitosan. The absence of the 1655 cm^{-1} group suggests the loss of free amines due to the bonding between chitosan, flavonoids, and PVA.

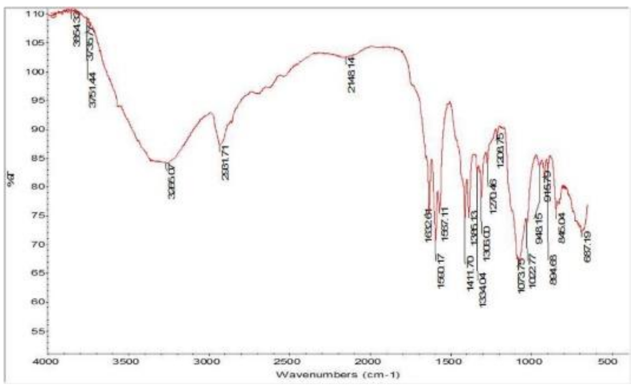
Additionally, the asymmetric vibrations of the methylene group in PVA were confirmed at the absorption peak of 2933 cm^{-1}, and the strain vibration of the C-O group in the acetyl group, which is part of the PVA backbone, was observed at 1096 cm^{-1}. In this study, these clusters were confirmed at peaks of 2931 cm^{-1} and 1073 cm-1, respectively. The presence of the saccharide structure in chitosan was also confirmed at wave numbers of 1208 cm^{-1} and 1022 cm^{-1}.

Furthermore, the modification of the film with sodium tripolyphosphate (STPP) led to the appearance of several peaks. An absorption peak at 915 cm^{-1} indicates the stretching of the P-O group from STPP. The presence of STPP as a crosslinking agent is confirmed by an absorption band at wave number 1238 cm^{-1}, indicating the P=O group. This crosslinking occurs due to the interaction between the negatively charged tripolyphosphate ion and the positively charged amine group in chitosan. This also observed in (Setiawan & Widiana, 2015). In the spectra shown in Figure 4.5, vibrations at a wavelength of 1270 cm^{-1} are also observed, suggesting the presence of the P=O group.

The spectrum analysis reveals the presence of several functional groups in the isolate. The aromatic C-H group is indicated by sharp absorptions at wave numbers 846.75 cm^{-1} and 790.81 cm^{-1}. Another group identified is the =C-H group, observed at wave number 3263.56 cm-1. The presence of a carbonyl group is confirmed at wave number 1681.93 cm^{-1}. Additionally, the aromatic –OH group is characterized by an absorption band at wave number 3558.67 cm^{-1}. The spectrum also exhibits absorption in the region of wave number 1045.42 cm^{-1}, suggesting the presence of a C-O alcohol group. Moreover, there is a possibility that the isolated structure contains aliphatic C-H groups, as indicated by absorptions at wave numbers 2989.66 cm^{-1} and 2875.86 cm^{-1} (Yadnya Putra et al., 2020).



(a)



(b)

Figure 3. Ch-PVA in various of binahong extract (a) 100 % and (b) 80%.

In Figure 3, it is evident that the addition of binahong extract resulted in the removal of several peaks between 1632 cm^{-1}590 cm^{-1} and peaks in the range of 1400-1200 cm^{-1}, as well as changes in the fingerprint regions around 845 cm^{-1}. The disappearance of these peaks suggests the formation of new bonds that dominate the spectral profile.

Water uptake. Water adsorption plays a crucial role in maintaining appropriate moisture levels within packaging films to preserve the quality of packaged products. This characteristic reflects the film's ability to swell when in contact with food, liquids, or when stored under high relative humidity conditions. The water adsorption capacity of films can also impact their mechanical properties. Additionally, water adsorption finds utility in certain applications, such as acting as adsorbent pads in the food industry.

Water permeability is an important parameter when evaluating plastic packaging. High water permeability can lead to dehydration, wilting, loss of freshness, and increased susceptibility to bacterial or fungal contamination.

The water uptake test provides valuable information about the amount of water molecules absorbed by the film. This is achieved by measuring the weight of the film before and after immersion in distilled water (aquadest). Based on the research findings, it is observed that the addition of 60% binahong extract resulted in the highest water uptake value, as depicted in Figure 4. The film with the best water uptake performance in this study was the one with 90% binahong leaf extract, showing a water uptake value of 491.52%. This indicates that the film exhibits the highest resistance to water permeability. As the concentration of binahong extract increases, the film becomes more resistant to water penetration.

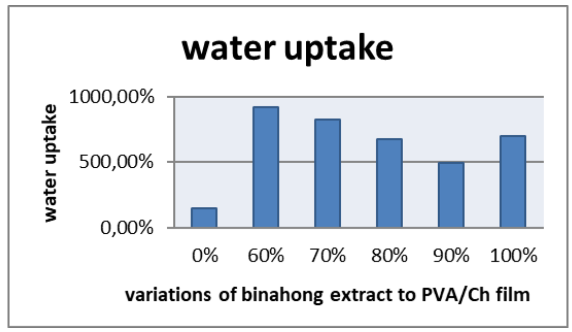


Figure 4. Water uptake of various binahong extract in film Ch-PVA.

In the research conducted by (Dyah Listianingsih, 2013), it was observed that the water resistance of the films was relatively low due to the hydrophilic nature of PVA. The presence of hydroxyl groups in PVA allows it to form hydrogen bonds with water molecules, leading to water retention on the film's surface (Gladis Aros Safitri & Eko Santoso, 2016).

On the other hand, chitosan exhibits low water solubility due to the presence of amine groups (-NH2) that are strongly bound to hydroxyl groups (-OH) (Svang-Ariyaskul et al., 2006). The water resistance of the composite film is significantly influenced by the composition of the materials used. In this study, the ratio of 0.2% chitosan to 0.3% PVA was used, with a 1:1 ratio.

The addition of binahong extract in higher concentrations resulted in increased water resistance of the film. This can be attributed to the presence of water-soluble components, such as flavonoids, in the binahong extract. Additionally, the extract contains organic solvent-soluble components like saponins and tannins. At certain compositions, the matrix formed by the binahong extract, chitosan, and PVA may become more compact, making it difficult for water to penetrate and break the bonds within the film.

The crystallinity of the film also plays a role in water permeation. Higher crystallinity can create pathways for water to permeate around the film, affecting the diffusion rate of water within the film matrix (J. Li et al., 2010). Therefore, the water resistance of the film is influenced by multiple factors, including the composition of the materials, the presence of soluble components, and film crystallinity.

In a study conducted by (Jipa et al., 2012), it was found that the water permeability of pure PVA was higher compared to the chitosan-PVA blend. This suggests that blending chitosan with PVA can enhance the film's resistance to water (Ren et al., 2015). Adding chitosan to the chitosan-PVA composite increases the hydrophilic properties of the film, as evidenced by the water vapor permeability values of PVA/CS-2 and PVA/CS-2.5 films (16.41 ± 2.66 g cm^{-1} $s^{-1}Pa^{-1}$ and 18.03 ± 2.82 g cm^{-1} s^{-1} Pa^{-1}).

The water uptake values in Figure 6 show that the addition of 60% and 70% binahong extract resulted in the highest values at 921.43% and 826.47%. This can be attributed to the hydrophilic properties of chitosan and PVA, which have the ability to absorb water in proportion to the weight of the film itself (Safitri et al., 2022). However, the addition of 100% binahong extract exhibited a different trend, possibly due to a less homogeneous mixing process, resulting in suboptimal bond interactions between the amine groups in chitosan, hydroxyl groups in PVA, and secondary metabolites in binahong extract.

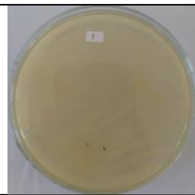
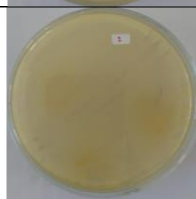
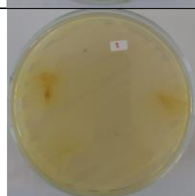
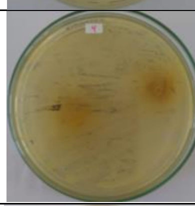
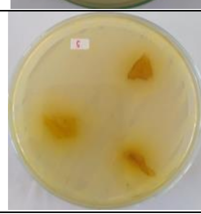
The control film (without binahong extract) had a yield at 146.55%. This lower result indicates a good density and a smooth film surface, indicating a well-mixed and homogeneous composite. Compared to SNI for non-biodegradable plastics, the water resistance can reach as low as 0.01%. Previous research has shown that biodegradable films exhibit water resistance up to a certain value, which varies depending on the specific type of film. According to (Costa-Júnior et al., 2009), Chi/PVA blend [25:75] before and after chemical crosslinking with 1% and 5% of glutaraldehyde content. Briefly, the observed pattern indicated an initial rapid mass uptake, usually in approximately 30 min, followed by mass stabilization over a 192 h period. Visual inspection of the samples also shows an appreciable volume increase. The results have revealed a strong influence of e-crosslinking on the swelling volume, from about 700% in chi/PVA sample before crosslinking, it dropped to 400% and 200%, with 1.0% and 5% glutaraldehyde. Meanwhile, based on previous research (Putri Utami Nur Zaisyah, 2022) the water resistance of biodegradable films reached 437.13% for biofilms from cassava rind. The similar behaviour from experiment (Bano et al., 2014) when Chitosan blends by various PVAL content is observed. The values for WVTR and WVP of membranes (Mem25, Mem50, Mem75) were 164.04, 144.44, 97.32 (×10−3) g h−1 m−2 and 1.87, 1.67, 1.09 (×10−6) g Pa−1 h−1 m−2, respectively. There was in inverse relationship between irradiation dose and (Water vapor transmission rate (WVTR) and water vapor permeability (WVP). This decrease in WVTR and WVP of chitosan membranes indicates that lowering the molecular weight of chitosan leads to more intermolecular interaction among polymer chains, resulting in tightening of the polymer network and lower permeability.

Antibacterial Test. Many studies have proposed an antibacterial mechanism in chitosan films. Along with the increasing of free amino groups on the chitosan film, its antibacterial properties are also getting solid. The positively charged amino group will form an electrostatic bond with the negative charge on the bacterial membrane. As a result, the protein that constitutes these microorganisms is leaked (Bonilla et al., 2014).

In previous studies conducted by (Dina Katrin et al., 2015), the antibacterial activity of binahong leaf extract (*Anredera cordifolia*) against Escherichia coli was tested using the disc diffusion method. This method is commonly used due to its ease of implementation. Initially, paper discs are immersed in the antibacterial fraction and then placed on agar media containing the cultured bacteria. After 24 hours of incubation, the inhibition zone around the disc is measured and observed. According to (Davis & Stout, 1971) the antibacterial efficacy based on the size of the inhibition zone is categorized into three zones: a very strong zone with a diameter of 20 mm or more, a strong zone with a diameter of 10-20 mm, and a weak zone with a diameter of 5-10 mm. A diameter of less than or equal to 5 mm indicates the weakest antibacterial activity. However, the disc method was found to be ineffective, leading to the replacement of disc paper with the film itself. The results of these observations are presented in Table 2, indicating that increasing concentrations of binahong extract expand the inhibition zone and thus enhance the antibacterial properties.

Table 2. Antibacterial Test.

Concentration of extract	24 hours of incubation	Diameter (mm)
0%		0

60%		0
70%		0
80%		10
90%		11
100%		12

An increase in the concentration of binahong extract results in higher inhibition of bacteria, primarily due to the presence of elevated levels of antibacterial agents such as flavonoids, saponins, and alkaloids in the film samples. These secondary metabolites have the ability to penetrate bacterial cells, form complexes with extracellular proteins, and activate enzymes. Flavonoid compounds, in particular, have been shown to have damaging effects on both Gram-positive and Gram-negative bacteria thereby inhibiting bacterial motility (Darsana, I.G.O. et al., 2012).

According to Rafique et al., various factors can affect the antibacterial activity of films, including the properties of the matrix film, manufacturing methods and conditions, and the specific bacteria being tested. When Ch-PVA is incorporated films firmly with the binahong extract, the antibacterial activity increases as the extract content increases. This can be attributed to two potential mechanisms: the film structure and the release behavior of binahong. The stronger the interactions between Ch-PVA incorporate with extract increase the solubility of films and slow down the release of antibacterial from the matrix into the bacterial suspension (Zhang et al., 2017).

Chitosan has been extensively investigated as an antimicrobial material, and its antibacterial activity is believed to be attributed to the presence of cationic amino groups. These amino groups have the ability to interact with the negatively charged surfaces of bacteria, leading to alterations in cell wall and membrane permeability. This, in turn, ruins cell metabolism, percolation of intracellular components, and ultimately results in fatality cell (J. Li et al., 2010).

Another reason that could impact antibacterial agent in the film is because Ch-PVA film forms agglomerates in the film surface as the electrostatic forces. These agglomerates restrict the contact between chitosan as an antibacterial agent and bacterial cells consequently it is hindered the diffusion of CS into the medium (Tahtat et al., 2011). (Zhang et al., 2016) suggested that the grainy features like seen in the film Ch-PVA 70% and 90%, such as scales or cracks, stimulate the formation of clumps when bacteria agglomerate and be drowned to the bottom, framing them less susceptive to growth.

This film has high potential to be developed with some room still left for future studies, including some characterization such as thermal stability, morphology properties, particle size analyser, mechanical strength, and hydrophilicity.

Conclusion

The best bacterial inhibition value on chitosan-PVA plastic was by adding 100% binahong extract by 12 mm. In the future the development of smart packaging with the addition of sensors to detect spoilage in food also needs to be developed so that this research can be applied in the future to detect spoilage in food more easily and practically

Conflict of Interest

The authors declare that there is no conflict of interest.

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