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Utilization of Local Wisdom of Antanan (Centella Asiatica) And Badotan (Ageratum Conyzoides L.) Plants for Anti-Bacterial Edible Film of Pumpkin Seeds

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ABSTRACT

Edible film is a thin layer made from consumable materials, formed on top of or placed between food components, functioning as a barrier to mass transfer such as moisture, oxygen, and solutes. It is made from edible biopolymers and food-grade additives. The development of edible film packaging in this study was carried out by utilizing pumpkin skin and seeds that have not been adequately used and by exploring the addition of natural antimicrobial compounds, namely antanan and badotan, to enhance the inhibitory effect of edible films made from pumpkin seeds that meet food standards.In this study, the extraction of yellow pumpkin seeds, antanan, and badotan was performed, followed by organoleptic and phytochemical tests. An edible film formulation was made from yellow pumpkin seeds, varied with antanan and badotan, and tested for moisture content, solubility, pH, and antibacterial properties. The characteristic test results showed that the badotan leaf simplicia was dark green, with a bitter taste and an astringent aromatic odor typical of badotan. The extract was blackish-green in color, with an aromatic odor typical of badotan, and a very bitter taste. Antanan leaf simplicia had a typical aromatic odor, an astringent taste, and was solid green in color. The antanan leaf extract had a typical antanan odor, a very bitter taste, and was thick green in color. Pumpkin seed simplicia had a typical pumpkin smell, an astringent taste, and was thick green in color. The pumpkin seed extract had a smell of ethanol solvent, a bitter taste, and was brownish-green and in the form of a thick liquid. The potential bioactive compounds in badotan leaves included alkaloids, steroids, phenolics, and saponins. Antanan leaves and pumpkin seeds contained flavonoids, alkaloids, steroids, phenolics, and saponins. The results showed that the edible film formulation of pumpkin seeds combined with badotan and antanan (1:2) appeared tighter and smoother and did not break. The formulation of pumpkin seeds and badotan (1:2) had a moisture content of 0.0380%, a solubility content of 0.2075%, and a pH of 5.8. The pumpkin seed and antanan (1:2) formulation had a moisture content of 0.0732%, a solubility content of 5.7269%, and a pH of 5.8. The edible film antibacterial activity test against Staphylococcus aureus used the disc diffusion method and obtained an inhibition zone of 1.8 mm for pumpkin seed edible film, 12.4 mm for pumpkin seed (1:2) edible film, and 17.6 mm for pumpkin seed (1:2) edible film.

Keywords: Edible Film, Pumpkin Seed, Antanan, Badotan, Inhibition

Introduction

An edible film is a thin layer made from consumable materials that is either formed over or placed between food components, serving as a barrier to mass transfer processes such as moisture, oxygen, and solutes (Agung et al., 2017). Edible films and coatings are produced from edible biopolymers and food-grade additives through various techniques, offering a sustainable packaging solution or protective layer for food surfaces. Biopolymers, including proteins, polysaccharides, lipids, or their combinations, can form coherent films and coatings (Ibtisam Kamal, 2019). The raw materials for edible films include proteins (polypeptides), carbohydrates (polysaccharides), and fats (lipids). These three components are thermoplastic polymers, known for their renewable and biodegradable properties (Marisa C. Gaspar, 2023).

According to screening studies by Rustina (2016) and Patel (2013), pumpkin seeds (C. moschata) contain carbohydrates, proteins, lipids, and compounds with antioxidant and antibacterial properties, such as phenolics and alkaloids (Ayu, 2020). Additionally, pumpkin skin contains pectin, which has been reported as an alternative material for bioplastic production (Mellinas et al., 2020). Pectin is a well-known gelling agent used in processed foods like jams and jellies. Research by Ulyarti et al. (2022) indicates that C. moschata skin extract contains flavonoids, alkaloids, saponins, and terpenoids, some of which possess natural antibacterial properties. Pumpkin is rich in starch, carbohydrates, protein, and fat (Ika Winda, 2015).

This study aimed to develop edible film packaging by utilizing underutilized pumpkin skin and seeds. Edible films can be enhanced with antibacterial compounds, a portion of which may migrate into the packaged food or product (Dwity and Rieny, 2021). Antanan (Centella asiatica) is commonly used in traditional Indonesian medicine for its wound-healing, antibacterial, antioxidant, and even anticancer properties. It has been shown to inhibit the growth of both gram-positive and gram-negative bacteria (Ella Yunita, 2020). The leaves of Antanan contain active compounds such as flavonoids, tannins, phenols, saponins, and steroids, which exert antibacterial effects by damaging bacterial cell walls (Siti Fatimah, 2022).

Badotan leaves (Ageratum conyzoides) are another wild plant widely used in traditional medicine by various ethnic groups in Indonesia and other countries. In Indonesia, A. conyzoides is commonly found in disturbed areas such as yards and gardens. While the medicinal uses of badotan have been widely reported, studies linking its use to microbial bioactivity in edible films are still limited. Previous research on edible films includes those made from refined carrageenan with added red galangal essential oil, producing an inhibition zone of 6.7 mm (Meivi, 2016); edible films with watermelon albedo pectin, sago, and garlic extract, producing a 5.5 mm inhibition zone (Fitria et al., 2020); and edible films with galangal essential oil, showing a 1.4 mm inhibition zone (Rina, 2018), among others, including formulations of edible films without added antibacterial compounds.

This research investigated the incorporation of natural antimicrobial compounds, specifically antanan and badotan, to improve the inhibitory properties of edible films derived from pumpkin seeds that meet food standards. Various characterization tests, such as thickness, solubility, vapor transmission, pH, and organoleptic properties, were performed. The most effective formulation of the pumpkin seed edible film was then evaluated for its antimicrobial activity with the inclusion of antanan and badotan.

Materials and methods

a. Equipment and Materials

The equipment used in this research includes a magnetic stirrer, digital oven, vacuum rotary evaporator, analytical balance, stirring rod, shaker, micrometer screw gauge, Erlenmeyer flask, measuring cup, vacuum, hotplate, thermometer, beaker, spatula, volume pipette, 20x20 cm glass mold, airtight container, sieve, blender, and scissors. The materials used comprise filter paper, Maizena flour, distilled water, 70% ethanol, glycerol ($C_3H_8O_3$), and clindamycin as a positive control. The test bacteria used were *Staphylococcus aureus*.

b. Research Procedure

1. Extraction

Pumpkin seeds were first washed and sorted to ensure good quality, then dried. The seeds were then mashed and filtered using a sieve. A 150-gram sample of *C. moschata* seeds was macerated for 3 days (72 hours) using 70% ethanol as the solvent. The macerate was stirred occasionally to ensure homogeneity and was thoroughly filtered. Filtration was then performed using filter paper. The resulting liquid extract was evaporated using a rotary evaporator (Depkes RI, 1986). The same procedure was applied to antanan and badotan. The symbiosis and extracts of pumpkin seeds, antanan, and badotan were then characterized.

2. Phytochemical screening

Flavonoids

A 1 mg amount of solid ethanol extract was placed on a drip plate. Then, 10 drops of ethanol were added and stirred using a spatula until dissolved. Next, 6 pieces of ribbon were added and 4 drops of concentrated HCl were added to the mixture. The emergence of yellow, blue, orange, or red color indicates a positive result (Octaviani et al., 2019).

Alkaloids

Weighed 4 mg of solid sample, dissolved it using 3 mL of methanol and 5 mL of ammonia at a pH of about 8-9, and then filtered the mixture. Next, 2 mL of 2M HCl solution was added to the filtrate and shaken. The resulting mixture was divided into 4 test tubes, with 5 drops added to each tube. Tube 1 contains a blank solution, while tubes 2, 3, and 4 were mixed with 1 drop each of Mayer, Wagner, and Dragendorff reagents, respectively. Positive results in this test are indicated by the presence of white, brown, or orange precipitate in the solutions (Suyani, 1991).

Steroids and Triterpenoids (Liebermann-Burchard Test)

A total of 1 mg of solid ethanol sample was placed on a drip plate, and 6 drops of anhydrous acetic acid were added. The mixture was stirred using a spatula until dissolved. Next, one drop of concentrated H_2SO_4 was added. A purple to orange color in the solution indicates the presence of triterpenoid compounds, while blue or green indicates the presence of steroid compounds (Suyani, 1991).

Phenolics

A 1 mg amount of solid sample was placed on a drip plate, and 10 drops of methanol were added. The mixture was stirred using a spatula until dissolved. Next, 6 drops of 5% $FeCl_3$ solution were added. The appearance of blue, green, purple, or reddish color indicates a positive result in the test (Suyani, 1991).

Saponins

A total of 1 mg of solid ethanol extract was placed in a test tube, and 5 mL of distilled water was added and shaken for 1 minute. If froth was formed, 4 drops of 1M HCl solution were added. If there was no froth, heating was continued for about 3 minutes. The mixture was then allowed to cool and shaken vigorously. The formation of stable froth within ±10 minutes indicates the presence of saponin compounds in the sample (Triwahyuono & Hidajati, 2020).

3. Preparation of Edible Film

Weigh 10 grams of corn starch and dissolve it in 100 mL of distilled water. Then, heat the solution and stir it on a magnetic stirrer at a temperature of 60°C until gelatinization occurs. After that, add 2.5 mL of glycerol as a plasticizer while stirring to ensure the starch particles and glycerol are well mixed. Finally, add the sample extract. The addition of the sample extract was done with three formulations:

- **Formulation 1**: pumpkin seeds and badotan (1:1)
- **Formulation 2**: pumpkin seeds and antanan (1:1)
- **Formulation 3**: pumpkin seeds and badotan (1:2)
- **Formulation 4**: pumpkin seeds and antanan (1:2)

The suspension was kept stirred until gelatinization occurred. The solution was then poured into a 20 x 20 cm glass mold. The mold containing the film solution was then dried in an oven at 45-50°C for 24 hours. The resulting edible film was tested for moisture content, solubility, and pH.

4. Antibacterial Activity Test of Edible Film Against *Staphylococcus aureus*

The antibacterial test of the seed extract edible film, and the mixture of both extracts, was carried out using the disc diffusion method. The test bacteria used was *Staphylococcus aureus*. The test medium used was MHA (Muller Hinton Agar), with a composition of 38 grams per liter. Bacterial colonies were prepared to a concentration equivalent to the McFarland standard, which is approximately 1.5 x 10^8 CFU/mL. Bacterial colonies were grown in MHA using the pour plate method. A 5 mm sample of the edible film was placed on the agar culture media and incubated at 37°C for 24 hours. The diameter of the resulting clear zone was measured using a millimeter scale. Clindamycin was used as a positive control, and sterile distilled water was used as a negative control (Esmail, 2021).

Results and Discussion

The results of the simplicia extraction from yellow pumpkin seeds, antanan, and badotan were tested organoleptically, including odor, taste, and color. The results are shown in Tables 1, 2, and 3.

Table 1 Organoleptic test results for simplisia and extract of Pumpkin seeds

No	Organoleptic test	Simplisia	Extract
1.	odor	Typical Yellow Pumpkin	Ethanol Solvent Odor
2.	taste	abusive	Abusive
3.	color	Deep Green	Dark brownish green

Table 2 Organoleptic test results for simplisia and extract of Antanan

No	Organoleptic test	Simplisia	Extract
1.	odor	Aromatic Characteristics	Typical of Antanan
2.	taste	abusive	very bitter
3.	color	deep green	thick deep green

Table 3 Organoleptic test results for simplisia and extract of Badotan

No	Organoleptic test	Simplisia	Extract
1.	odor	Aromatic Characteristics	Aromatic Characteristics
2.	taste	bitter	very bitter
3.	color	dark green	blackish green

(a) (b) (c)

Figure 1. Simplisia badotan (a), simplisia antanan (b), simplisia Pumpkin seeds (c)

(a) (b) (c)

Figure 2. Extraction Badotan (a), Extraction antanan (b), Extraction Pumpkin seeds

Phytochemical analysis revealed the presence of secondary metabolites, including flavonoids, alkaloids, steroids, phenolics, and saponins, in ethanol extracts from antanan plants and yellow pumpkin seeds. However, ethanol extracts from badotan contained alkaloids, steroids, phenolics, and saponins but tested negative for flavonoids. Flavonoid levels were assessed using Shinoda reagents, which caused the solution to turn orange due to the formation of complex compounds between magnesium ions and phenoxy ions in the flavonoids. The reduction of flavonoids in the extract when treated with concentrated Mg^{2+} and HCl led to the creation of an orange-colored complex $[Mg(OAr)_6]^{4-}$ (Dyera, 2020).

The ethanol extracts of antanan and yellow pumpkin seeds were confirmed to contain alkaloids, as evidenced by the formation of a white precipitate with Mayer's reagent, brown deposits with Wagner's reagent, and orange deposits with Dragendorff's reagent. These precipitates result from the formation of potassium-alkaloid complexes, where the nitrogen atoms in the alkaloids, which have free electron pairs, bind to K^+ ions in the alkaloid reagents (Farida, 2021).

In steroid testing, the ethanol extract turned a bluish-green color, likely due to the oxidation of the steroids, leading to the formation of conjugated bonds (Farida, 2021). Additionally, the ethanol extracts of badotan, antanan, and yellow pumpkin seeds turned purple, indicating the presence of phenolic compounds. This color change occurs due to the formation of complex compounds between ferric ions and phenoxide ions.

The saponin compounds were tested using the "Forth" method, which resulted in the generation of stable foam with a height of 2 cm, lasting approximately 10 minutes. The formation of stable foam is attributed to the glycosides' ability to produce foam in water and then undergo hydrolysis into glucose and other compounds.

Table 4. Phytochemical Analysis

Bioactive compounds	Badotan	antanan	Pumpkin seed
Flavanoid	-	+	+
Alkaloids	+	+	+
Steroids	+	+	+
Phenolic	+	+	+
Saponins	+	+	+

Formulation 1 is pumpkin seeds and badotan (1:1), formulation 2 is pumpkin seeds and antanan (1:1), formulation 3 is pumpkin seeds and badotan (1:2), and formulation 4 is pumpkin and anthuran seeds (1:2). From the surface results, it appears that the formulation of the film made from pumpkin seeds combined with badotan and antanan (1:2) is tighter and smoother, and does not break.

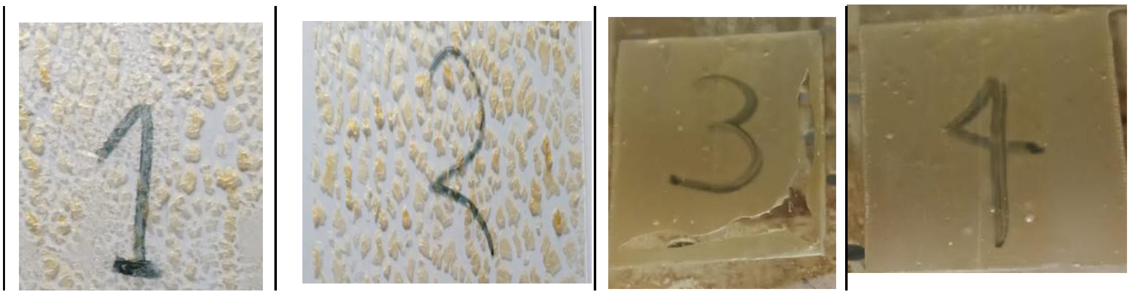

Figure 3. Formulation of edible film

The results of the tests for moisture content, solubility, and acidity (pH) are summarized in Table 5. According to SNI 01-3751-2009, the maximum allowable moisture content for edible film is 14.5%. All tested edible film formulations met this standard. Solubility is crucial for edible films to ensure they dissolve appropriately when consumed and to assess their biodegradability if used for packaging. Santoso et al. (2011) note that an increase in solution molecules can strengthen the film matrix, resulting in a more compact and resilient film structure that resists water damage.

The acidity level, measured by pH, indicates the solution’s acidity. pH measurement helps ensure that the edible film’s acidity aligns with the physiological pH range of oral environments, preventing irritation during use. The pH values of the edible films ranged from 5.0 to 7.6. Edible films are a sustainable technology that uses edible materials for packaging other foods, integrating packaging with food through effective material design (Azeredo, 2022). The pH measurements confirm that the edible films fall within the physiological pH range of 5.5 to 7.9 (Barman and Umesh), with the average pH being 5.8, thus meeting the required standards.

Table 5. The results of the moisture content, solubility, and acidity (pH)

	Kadar Air (%)	Kadar Kelarutan (%)	pH
Formulasi 1	0,0627	0,2313	5,8
Formulasi 2	0,0529	0,4221	5,8
Formulasi 3	0,0380	0,2075	5,8
Formulasi 4	0,0731	5,7265	5,9

The results of the antibacterial test on the edible film formulations, conducted using the diffusion method with Muller Hinton Agar (MHA) and testing against *Staphylococcus* bacteria, revealed the following: The edible film made from yellow pumpkin seeds showed a bacterial inhibition zone of 1.8 mm. The edible film with yellow pumpkin seeds combined with antanan (1:2) exhibited an inhibition zone of 12.4 mm, while the yellow pumpkin seeds combined with badotan (1:2) had an inhibition zone of 17.6 mm (Figure 4).

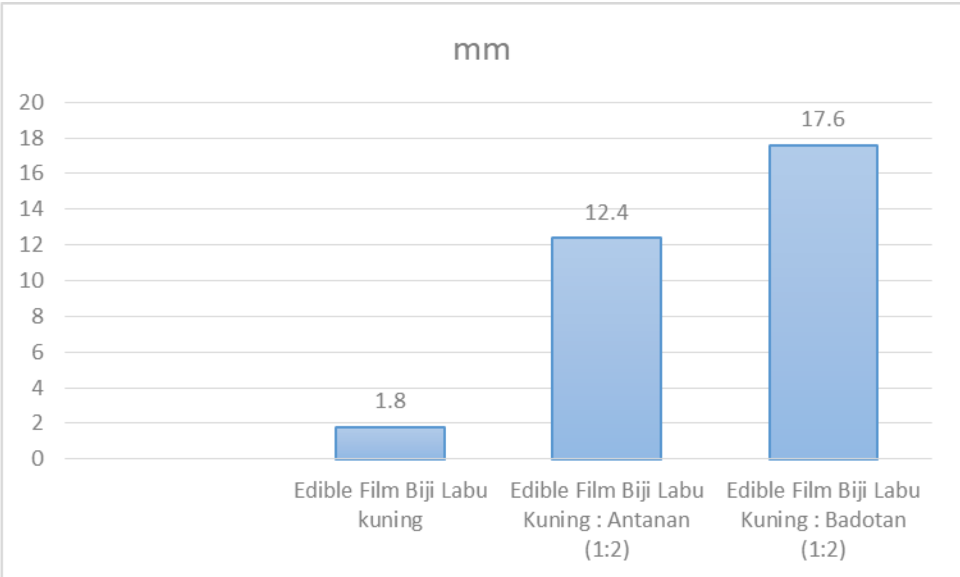

Figure 4. Anti-bacterial test for Staphylococus

CONCLUSION

The study revealed that badotan leaves are rich in bioactive compounds such as alkaloids, steroids, phenolics, and saponins. In comparison, antanan leaves and pumpkin seeds contain flavonoids, alkaloids, steroids, phenolics, and saponins. The results indicated that the edible film formulation combining pumpkin seeds with badotan and antanan (1:2) exhibited a tighter and smoother texture and was resistant to breaking. Specifically, the formulation of pumpkin seeds and badotan (1:2) had a moisture content of 0.0380%, a solubility of 0.2075, and a pH of 5.8. The pumpkin seed and antanan (1:2) formulation showed a moisture content of 0.0732%, a solubility of 5.7269%, and a pH of 5.8. The antibacterial activity test against *Staphylococcus aureus*, performed using the disc diffusion method, revealed an inhibition zone of 1.8 mm for the pumpkin seed edible film, 12.4 mm for the pumpkin seed and antanan (1:2) edible film, and 17.6 mm for the pumpkin seed and badotan (1:2) edible film.

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