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Original Article

FORMULATION AND EVALUATION OF EDIBLE FILM BETEL (*PIPER BETLE* L.) LEAVES EXTRACT AS MOUTH FRESHNER

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ABSTRACT

Objective: The purpose of this study is to create and assess edible film formulations from betel (*Piper betle* L.) leaf extract that may be used to treat *Streptococcus mutans* bacteria-caused halitosis.

Methods: Using ethanol as a solvent, the extraction process was employed to produce a concentrated extract of betel leaf. To find the minimum dose that could suppress bacteria development, the antibacterial activity of the ethanol extract of betel leaf was studied on *streptococcus* bacteria. The dosage of the betel leaf ethanol extract formula and the creation of edible film formulations are then based on the results of the antibacterial activity and evaluation of edible films.

Results: The ethanol extract of betel leaf and edible film formulations can both prevent the growth of *S. mutans* bacteria, according to the results of the antibacterial activity test. The evaluation's findings demonstrate that the three edible film formulations of betel leaf ethanol extract satisfy the Indonesian National Standard's requirements (SNI). When compared to the other formula. Formula 3 demonstrated the strongest antibacterial activity.

Conclusion: According to the study's findings, it is possible to apply the ethanol extract of betel leaf in the form of an edible film. *S. mutans* bacteria can have their growth inhibited by betel leaf ethanol extract. The betel leaf ethanol extract edible film formula has satisfied the criteria, and formula 3 was the best edible film formula.

Keywords: Betel (*Piper betle* L.) leaf, Antibacterial activity, Edible film

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INTRODUCTION

Low self-esteem and a loss of self-image are the psychological effects that follow. Whether the offensive substance is oral or non-oral in origin, poor breath that emanates from the oral cavity is referred to as having halitosis in general. The mouth and teeth are bodily parts that play a significant role in maintaining overall health [1]. Bacteria tend to congregate in the oral cavity. Bacteria that were once commensal can develop into pathogens and cause infection if there is a decline in bacterial immunity. *Streptococcus mutans* is one of the microorganisms frequently detected in the mouth cavity [2, 3]. One of the plants with anti-halitosis qualities is betel (*Piper betle* L.). Essential oils made from betel leaf extract primarily consist of phenol derivatives, specifically betel phenol and chavicol. The betel leaf's phenolic components have potent antibacterial and antifungal effects and are excellent at preventing the growth of a variety of bacteria. The concentration and length of exposure have a significant impact on the efficacy of antiseptic chemicals. The antiseptic component will be more potent at higher concentrations and for longer exposure times. Other studies findings have demonstrated that chavicol, which gives betel (*Piper betle* L.) leaves their distinctive smell, has five times more bacteria killing power than phenol [3, 4]. According to Anang Hermawan's (2007) research findings, *S. mutans* bacteria can be strongly inhibited from growing when betel leaf extract is used. Betel leaf is traditionally used as an anti-halitosis by crushing 2-4 leaves in the mouth or with hot water. It is used to rinse after cooling. This preparation needs to be freshly produced before use. It cannot be used again for 24 h. The volatile oil component that is effective as an ant halitosis is volatile in hot water. The betel leaf extract, which was macerated with 96% ethanol, was made into edible film preparations to increase the volatile oil's stability, practicality, usability, and public acceptance. Betel leaf ethanol extract can be employed as an active component in the creation of edible films [5].

MATERIALS AND METHODS

Materials

Betel leaf was the plant utilized in this study. *Streptococcus mutans* ATCC 25175 was the strain of bacteria in this study. Materials utilized include ethanol (96%), hydrochloric acid, toluene, chloroform, ammonia, Dragendorff, Mayer, and Liebermann-Burchard reagents, was analytical grade. While sucralose and menthol was pharmaceutical grade. Nutrition agar and Muller Hilton Agar was biological grade.

Identifying plants

Betel leaf was tested to determine its composition. The Plant Taxonomy Laboratory, FMIPA UNPAD's Department of Biology, does plant identification [6].

Plant preparation

The plant material utilized was betel leaf, which was sourced from the adultery area of Ciamis, West Java. It was subjected to a procedure that included wet sorting, washing, cutting, drying, and dry sorting as well as botanical determination to test the authenticity of natural elements [6].

Testing the simplicial properties

Determining the water and ash contents of simplicial were the qualities that are tested [6, 7].

Phytochemical screening

The alkaloids, flavonoids, tannins, saponins, and steroids, terpenoids are examined during the phytochemical screening of samples to ascertain the type of chemical compounds present in betel leaf extract [7].

Extract preparation

The maceration method is used to get the extract from 500 g of powdered dry betel leaf. After adding 96% ethanol to the sample in the macerator, it was occasionally agitated to ensure proper mixing. The maceration procedure was left to stand for 24 h before being collected, filtered, and replenished with fresh ethanol three times per day. After being collected, the macerate is circulated in a rotary evaporator to produce a thick liquid [7].

Test for antibacterial activity in betel leaf extract

By using disc diffusion to test for antibacterial activity against *S. mutans* bacteria, the diameter of the inhibition zone of the betel leaf

ethanol extract was evaluated at four different doses, including 0, 2, 4, and 6% [7]

Formula for an edible betel leaf film

Utilizing a magnetic stirrer, dissolve Na-CMC in a hot solution and mix until homogenous. Add PEG 400 after homogenization and mix one more time. Stir in the menthol and sucralose until well combined. The extract is then added at each formula's specified concentration. Put the edible film component combination in a petri dish, and then bake it to dry it out. Cut the 2x3 cm edible film compositions after drying [7, 8].

An evaluation of edible betel leaf film

Organoleptic, pH, thickness, weight uniformity, multiple resistance, disintegration, and dissolution time were among the evaluations conducted [8, 9].

Table 1: Formula for an edible betel leaf film

Formula (% b/v)					
	F0	**F1**	**F2**	**F3**	**Function**
Extract ethanol betel leaf	0	2	4	6	Active substance
Na-CMC	3	3	3	3	Basic of Edible film
PEG 400	0.8	0.8	0.8	0.8	Plasticizer
Sucralose	0.15	0.15	0.15	0.15	Sweetener
Menthol	0.2	0.2	0.2	0.2	Freshener
Aquadeion add	100	100	100	100	Eluent

Mechanical edible film test

The edible film is put through mechanical tests that measure its tensile strength, elasticity, and modulus of elasticity. The LIPI UCT 500 tool was used to conduct the mechanical test of the edible film [8, 9].

Test for antimicrobial activity in edible film preparations

After measuring the diameter of the inhibition zone of betel leaf edible film preparations at different concentrations, including 0, 2, 4, and 6%. The test was proceeded by analysing the antibacterial activity of edible film preparations by agar diffusion against *S. mutans* [7-9].

RESULTS

An edible film used as an anti-helianthus was created using an ethanol extract of betel leaf. According to the study's findings, the inhibition zone value obtained at a concentration of 2–6% indicated that the ethanolic extract of betel leaf had antibacterial activity against the *Streptococcus mutans* bacterium. The concentration of betel leaf employed in the creation of the formulations for edible films was determined by the findings of this antibacterial activity test. The betel leaf edible film test findings revealed outcomes that complied with the specifications for the creation of edible films.

Table 2: Antibacterial activity test results

Formula	Inhibition zone±SD
Formula 0	0
Formula 1	15.32
Formula 2	17.62
Formula 3	

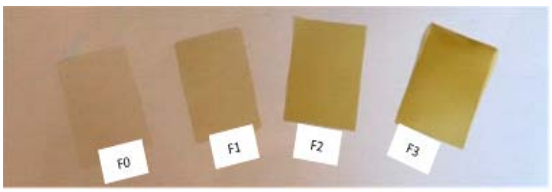

Fig. 1: Edible film results for each formula

Table 3: Organoleptic test results for edible films

Organoleptic	F0	F1	F2	F3
Colour	Transparent	green	Brownish green	Deep green
Smell	menthol	Betel Leaf	Betel Leaf	Betel Leaf
Flavor	Sweet and cooling but not	Sweet and cooling but	Sweet and cooling but not	Pleasantly bitter, reviving,
Form	bumpy	not bumpy	bumpy	and lump-free
	thin layer	thin layer	thin layer	thin layer

Table 4: Edible film pH test results

Formula	Average pH of preparation
Formula 0	7.14
Formula 1	7.27
Formula 2	7.28
Formula 3	7.28

Table 5: Weight uniformity results

Formula	Average dosage (mg)±SD
Formula 0	54.71
Formula 1	
Formula 2	
Formula 3	

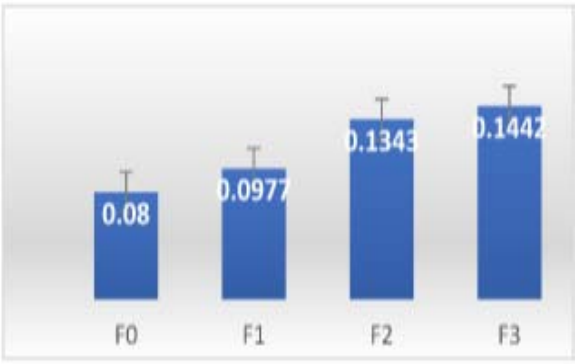

Fig. 2: Edible film thickness results

Table 6: Destruction test and dissolving results

Formula	Average disintegration time (seconds)±SD	Average dissolving time (seconds)±SD
Formula 0	18.00	
Formula 1	20.67	30.00
Formula 2	28.00	44.67
Formula 3	38.33	57.00

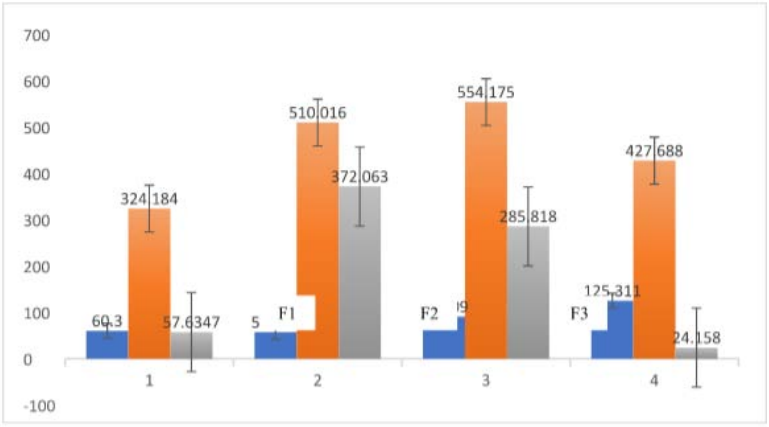

Fig. 3: Mechanical test results of edible film

Table 7: Antibacterial test for edible films

Formula	Zona Hambat
Formula 0	0.00
Formula 1	3.03±0.028
Formula 2	7.41±0.015
Formula 3	11.61±0.017

DISCUCION

The analysis' findings demonstrated that the material utilized was genuine betel leaf. The betel leaf was acquired and subjected to wet sorting, washing, cutting, and drying in the sun. The sample utilized in this investigation weighed approximately 2.5 kg. Simplicia is blended after being dried. The dried simplicial was macerated at room temperature for three consecutive 24 h periods, stirring every hour or so. To create a concentrated extract, the extracted filtrate was concentrated using a rotary evaporator. 11.89% of the betel leaf extract was produced. Both simplicial and betel leaf extracts contain alkaloids, phenols, saponins, steroids/terpenoids, and flavonoids, according to the results of the phytochemical screening. The results of the simplicia parameter test indicated that the ash and water content were 4 and 12.7%, respectively. This complies with the requirements of the Indonesian Medical Materials Volume IV, namely the limits on the ash content and water content of not more than 14% and 10%, respectively [6, 7]. *Streptococcus mutans* bacteria were used in a disc diffusion antibacterial efficacy test. The betel leaf extract includes secondary metabolites, specifically flavonoids and phenols, which have antibacterial properties, causing a clean zone to form around the paper disc. The diameter of the inhibition zone of betel leaf extract at a concentration of 6 percent produced the biggest inhibition zone, measuring 18.3±0.011 mm, and the lowest inhibition zone, measuring 15.32±0.045 mm, at a concentration of 2 percent [6].

By adding betel leaf extract as an active ingredient, edible film formulations are made. Employing the plasticizer PEG 400 and a polymer film called Na-CMC. There are three different concentrations of betel leaf extract that are used: 2%, 4%, and 6%. An organoleptic test, pH test, thickness test, weight uniformity test, destruction test, dissolving test, multiple resistance test, and edible film mechanical test are all used to evaluate betel leaf ethanol extract edible films [7, 8].

The edible film formulations of betel leaf extract during organoleptic testing vary. This is because each formula uses a different concentration of betel leaf extract; the more concentrated the colour produced, the greater the extract concentration. Each formula, including colour, smell, taste, and shape, demonstrates that the edible film preparation does not alter during the storage procedure [9, 10].

The pH test of the edible film was conducted to ascertain the preparation's pH and compare it to the mouth's pH. In this study, the pH of the edible film matches the pH of the mouth, preventing irritation of the oral mucosa if the edible film is swallowed [9].

According to the thickness test, the betel leaf edible film's findings meet the standards because its thickness is less than 0.25 mm. The number of total solids included in the dried edible film increases as betel leaf extract concentration increases, resulting in a thicker film [9, 10].

The weights of the four edible film compositions vary. The edible film falls within the 102-132 mg, susceptible range in the weight uniformity test [9, 10]. When the edible film starts to totally dissolve when it meets water or saliva, this is referred to as the disintegration period. When this happens, the edible film is said to dissolve. The four formulas in this investigation produced results that fall under the 1-minute time limit [11, 12]. The multiple resistance test was used to evaluate the film's mechanical toughness. The multiple resistance values for all formulas were greater than 300 times the susceptible requirement, indicating that the betel leaf film had good fastness and was neither damaged nor split [12, 13]. Tensile strength, percent elongation, and modulus of elasticity experiments on mechanical characteristics were used to define parameters for film properties. Tensile strength is a metric for film toughness; it can be defined as the highest tensile strength that can be applied to a film without causing it to tear or break. The tensile strength test findings demonstrate that the tensile strength tends to increase as more extracts are applied. The strength of the edible film to withstand physical damage during packing is referred to as its tensile strength. Up until the film breaks, the % elongation represents the largest length change during stretching. There is a correlation between the percent elongation and the tensile strength test: the lower the tensile strength, the higher the percent elongation produced [13, 14].

The inclusion of a plasticizer is crucial because it makes the film more flexible and prevents it from becoming brittle. Although the

modulus of elasticity is a fundamental gauge of a film's stiffness, its main function is to estimate the stiffness of the final product. The findings of betel leaf extract's decreased elastic modulus in edible film preparations can be influenced by the thickness of the film; the thinner the film, the lower the elastic modulus produced [13-15]. Based on the results, it can be concluded that larger diameters are created when edible film compositions contain higher concentrations of betel (*Piper betle* L.) leaf extract. Formula 1 considers it weak; Formula 2 considers it moderate, and Formula 3 considers it powerful.

CONCLUSION

The ethanol extract of betel leaf can be used to create edible films, and that formula 3 of the edible film made from the extract of betel leaf has good qualities. Formula 3 can inhibit the growth of *Streptococcus mutans* bacteria, with a concentration of 6% producing the largest diameter of the inhibition zone.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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