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Formulation of Edible Film Preparation from Musk Orange Essential Oil (*Citrus Microcarpa* Bunge) in Inhibiting the Growth of *Streptococcus mutans* Bacteria and *Candida Albicans* sp Fungus

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Abstract: This study aims to describe the formulation of edible film preparations from musk orange essential oil (*Citrus Microcarpa* Bunge) in inhibiting the growth of *Streptococcus mutans* and *Candida Albicans* sp. Fungus. The data collection technique uses laboratory-based experimental testing. Musk lime plants were taxonomically identified at the Herbarium Medanase (MEDA) laboratory, USU Campus, Medan, then processed using the steam distillation method to produce essential oils. The essential oil activity test uses concentrations (1%, 2%, 3%, 4% and 5%) to test its antibacterial and antifungal activity. Musk orange essential oil made in the form of an edible film was then tested for its physical properties as well as antibacterial and antifungal activity against the fungi S. mutans and C. albicans. The yield of essential oils was 0.93539gr/ml from 15kg of 8.5ml sample. The test results of the edible film preparation of the essential oil of musk orange have a distinctive aroma of fresh menthol and musk orange, orange color, smooth texture, sweet taste. Testing the ability of edible film preparations from essential oils has the highest inhibition zone results at a concentration of 4%, 5% which has natural antimicrobial substances. The yield of essential oils was 0.93539gr/ml from 15kg of 8.5ml sample. Essential oil activity tests produced the highest inhibition zones in bacteria and fungi at concentrations of 4% and 5%. The test results of the edible film preparation of the essential oil of musk orange have a distinctive aroma of fresh menthol and musk orange, orange color, smooth texture, sweet taste. Testing the ability of edible film preparations from essential oils has the highest inhibition zone results at a concentration of 4%, 5% which has natural antimicrobial substances.
Keywords: Edible film of essential oils; musk orange; growth of bacteria and fungi

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INTRODUCTION

The mouth is a vital organ in the body, requiring special care. It contains several vital organs, including the teeth, tongue, and more. We must maintain the cleanliness of everything in our mouths to prevent unwanted odors and discomfort. A healthy oral cavity allows for effective communication. Halitosis (bad breath) negatively impacts all of these functions and can even trigger stress (Wida Ningsih & Afdhil Arel, 2022).

Bad breath is caused by bacterial and fungal infections that attack the oral cavity, triggering tooth decay and making the breath smell bad. One of the bacteria that causes bad breath is *Streptococcus mutans*, a pathogenic bacterium that causes dental caries. According to researchers (Setiani, 2020). *Streptococcus mutans* is a microorganism that causes bad breath. The bacteria are gram-positive, non-motile, and anaerobic, spherical and chain-shaped, but do not form spores. These bacteria can live at temperatures of 14-4°C and thrive in environments rich in sucrose but can cause the formation of an acidic environment in the oral cavity. Another cause of bad breath is the fungus *Candida albicans*, a fungus from the yeast group. In the oral cavity, *Candida albicans* can cause thrush that causes bad breath. *Candida albicans* lives as a saprophyte and can turn into a pathogen. *Candida albicans* in the oral cavity occurs through several stages: acquisition of *Candida albicans* from the environment, growth stability, attachment, and penetration of *Candida albicans*. There are many ways to prevent bad breath, one of which is by using plants (Drasar, 2003).

Plants that have the potential to inhibit the growth of *Streptococcus mutans* and *Candida albicans* are musk oranges. Musk oranges can be used to treat bad breath because musk oranges (*Citrus microcarpa* bunge) contain 6-8% citric acid. Researchers (Chodijah et al., 2019) have conducted extensive research to find alternative medicines to replace existing chemicals. Medicinal plants have become the subject of various studies, namely the musk oranges plant (*Citrus microcarpa* Bunge) is one of the plants that has antibacterial properties as a medicine. The inhibition zone produced from this essential oil has a strong ability of 13mm in overcoming the growth of bacteria and fungi. In general, musk oranges are preferred because of their unique taste and high vitamin C content, and balanced calcium levels, making them a frequent consumer favorite. The peel of musk oranges can produce essential oils. The essential oil of musk oranges (*Citrus microcarpa* Bunge) is one of the plants that can be used to treat bad breath because it contains compounds in musk limes such as ascorbic acid. Because the cassava plant has a distinctive aroma and contains good ingredients for the body, this plant is often used as a mouth freshener supplement, one of which is in the form of edible film (Viani, 2024)

Edible film is a thin layer made of edible material, formed on top of food components that function as a barrier to mass transfer such as water, oxygen, and dissolved substances. This edible film can also be stated as one of the trends in the health industry, both the food industry and material science due to the combination of technological innovation and sustainability such as we can use it directly without having to throw away the packaging because this edible film is generally made from organic materials such as fat, protein, and carbohydrates (starch/non-starch). This thin packaging (Edible film) is antibacterial that can reduce, inhibit or slow the growth of pathogenic microorganisms. This edible film has a very thin layer, made of hydrophilic components such as protein, carbohydrates, and fat or a combination of the three. Edible film is a type of formulation that can dissolve immediately after contact with saliva. Once dissolved, the active ingredients in it are immediately released and provide pharmacological effects. (Putra et al., 2017).

A lot of research has been done on edible films made from various types of starch raw materials and essential oils. One of the edibles of the film is one made from tapioca starch and white turmeric essential oil, which can suppress the bacteria Pseudomonas putida. This study has a clear zone of about 9 mm at a concentration of 2% (Putra et al., 2017). According to research that has been carried out, testing the antibacterial activity of citronelal essential oil from kaffir lime fruit (Citrus hystrix DC.) at a concentration of 4%, 6% 8% refers to a clear area against Streptococcus mutans bacteria while the clear zone of 8.00 mm is found at a concentration of 4%, 8.06 mm with a concentration of 6% and 8.73 mm with a concentration of 8% (Ningsih & Arel, 2023). However, testing for making edible films from musk orange essential oil has never been carried out, therefore researchers are interested in conducting a test with the aims to describe the formulation of edible film preparations from musk orange essential oil (*Citrus Microcarpa* Bunge) in inhibiting the growth of *Streptococcus mutans* and *Candida Albicans* sp.

METHOD

The method used in the study was an experimental method using a Complete Random Design (RAL) which consisted of five treatments and was repeated three times so that there were a total of 5 experimental units. Here is the treatment: A1 = 1 % musk orange leaf essential oil A2 = 2 % musk orange leaf essential oil A3 = 3 % musk orange leaf essential oil A4 = 4 % katsuri orange leaf essential oil A5 = 5 % musk orange leaf essential oil

This study also uses a disc diffusion method test with some concentration of essential oil from musk orange (*Citrus microcarpa* bunge) which will be formulated into an edible film to inhibit the growth of bacteria *Streptococcus mutants* and *Candida albicans* fungi are among them with concentrations as explained above which are made 3 times. For negative control use an edible film formulation without the addition of essential oils and for positive control use Gofress xylitol.

This data collection technique can be used with laboratory-based experimental testing. This experimental method was carried out to find out if the product given by the researcher was suitable. In addition, a table was also used to see the results of observations in the inhibition zone using musk orange peel essential oil (Citrus microcarpa bunge) which was made in the form of an edible film and tested for physical properties as well as antibacterial and antifungal activity against *Sterptococcus mutans* and *Candida albicans fungus*.

The ingredients used include: Musk orange essential oil, corn starch, Sorbitol 70%, Na. Saccharin, Menthol, Nipagin, Nipasol, Orange essence, Distilled water (aquades). Bacterial and fungal breeding media, namely Mueller Hinton Agar (MHA) and Pda (Potato dextrose agar media), hydroxypropyl methyl cellulose (HPMC) (Phapros), McFarland standard 1.5 x 108 CFU/mL, DMSO solvent, Streptococcus mutans bacterial culture and Candida albicans fungus.

RESULTS AND DISCUSSION

Characteristics of Musk Orange Essential Oil (*Citrus macrocarpa* Bunge)

Essential Oil from the bark of the musk orange (*Citrus microcarpa* bunge) is obtained from the Musk Orange plant (*Citrus macrocarpa* Bunge) which comes from the family Rutaceae. This plant has been taxonomically identified at the Medanase Herbarium Laboratory (MEDA) Jalan Bioeknologi No.1 USU Campus, Medan. The identification results show that musk oranges belong to the Kingdom Plantae, Spermatophyte Division, Class Dicotyledoneae and Order Sapindales, with the local names of the plant known as musk orange, genus Citrus and species Citrus microcarpa Bunge.

The results of the process of making musk orange essential oil in this study obtained a yield of 0.93539 gr/ml from a sample of 15 kg of musk orange, producing 8.5 ml of oil. According to the opinion of Ikarini et al., (2021) that the results of the calculation of essential oil yield can be influenced by the factors of distillation time, temperature, type and quality of the ingredients used. So that the results of this distillation process produce essential oils that have the characteristics of essential oils from musk oranges (*Citrus microcarpa* bunge).

Identification of Musk Orange Essential Oil (*Citrus microcrpa* Bunge)

The identification of musk orange essential oil (*Citrus macrocarpa* Bunge) includes, organoleptic test, odor, color, spot, time test of essential oil type, solubility test in ethanol. The results of the organoleptic test of musk orange essential oil (*Citrus macrocarpa* Bunge) can be seen in the table below.

Table 1. Organoleptic test results of mustard orange essential oil (*Citrus microcarpa* Bunge)

No	Test Parameters	Result
1	Aroma (construction)	Special essential oil of mushroom oranges
2	Color	133 Apha, 0.2 Red, 3.4 Yellow, 1.0 Gadner (Kuning)
3	Patches	Spots fused with filter paper
4	Density	0.93539 gr/ml
5	Solubility in Ethanol	Soluble in ethanol

This organoleptic test aims to see the physical appearance of the essential oil by observing the Smell (Aroma), Color, Spot, Density and Solubility in ethanol from the preparation of the Musk Orange Essential Oil preparation. In the organoleptic testing of musk orange essential oil (*Citrus microcarpa* bunge) it was obtained that the oil has a distinctive aroma of musk orange peel, has a yellow color with (color code 133 Apha, 0.2 Red, 3.4 Yellow, 1.0 Gadner), oil spot test that is fused with filter paper.

The density of musk orange essential oil (*Citrus microcarpa* bunge) was calculated using a picnometer with a volume of 10ml, resulting in a density value of 0.93539 g/ml. This value is close to the SNI 06-2385-2006 standard, which is between 0.9357-0.9603g/ml at a temperature of 95-100°C which shows that the essential oils obtained have good quality and are pure essential oils. (Annisa et al., 2020). The results of the solubility test in ethanol of musk orange essential oil (*Citrus microcarpa* bunge) were clear and well soluble in ethanol. The solubility in ethanol is a parameter that determines the quality of essential oils. Essential oils that are perfectly soluble in ethanol exhibit their constituent compounds that are higher polar (Loppies et al., 2021).

Results of Gram Staining of *Streptococcus mutans* bacteria and *Candida albicans fungus*

Gram Staining Results of *Streptococcus mutans* Bacteria and *Candida albicans Fungus* have the characteristic of being coconut-shaped (round or ovate) and generally chain-shaped. *Streptococcus mutans* is a gram-positive, non-motil, and anaerobic bacterium (Hasanuddin & Salnus, 2020). Candida *albicans* fungus has the characteristic of a round or oval shape, is small, thin-walled, and can form pseudohyphae (have pseudohyphae). According to research by Pasaribu *et al*., (2019) The morphological characteristics of *Candida albicans* are dysmorphic fungi because of their ability to grow in two different forms, namely as sprouting cells that will develop into blastospores and produce sprouts that will form pseudo-hyphae. This difference in shape depends on external factors that affect it. Yeast cells (*blastospores)* are round, oval or oval spherical in size with sizes of 2-5μ x 3-6μ to 2-5.5μ x 5-28μ.

Antibacterial Activity of Musk Orange Essential Oil (*Citrus microcarpa* bunge) against *Streptococcus mutans* Bacteria.

Based on the results of the Antibacterial Activity Test of essential oils at the Microbiology Research Laboratory of the State Islamic University of North Sumatra. Testing the antibacterial activity of musk orangeessential oil (*Citrus microcarpa* bunge) against *Streptococcus mutans* bacteria, using the disc diffusion method by means of the concentration of each oil being absorbed on a disc paper which will produce an inhibition zone in the form of a clear area in each repetition. This method is the choice in activity testing because it has the advantage of a simple procedure (easy and practical) and is often used (Jamila et al., 2022).

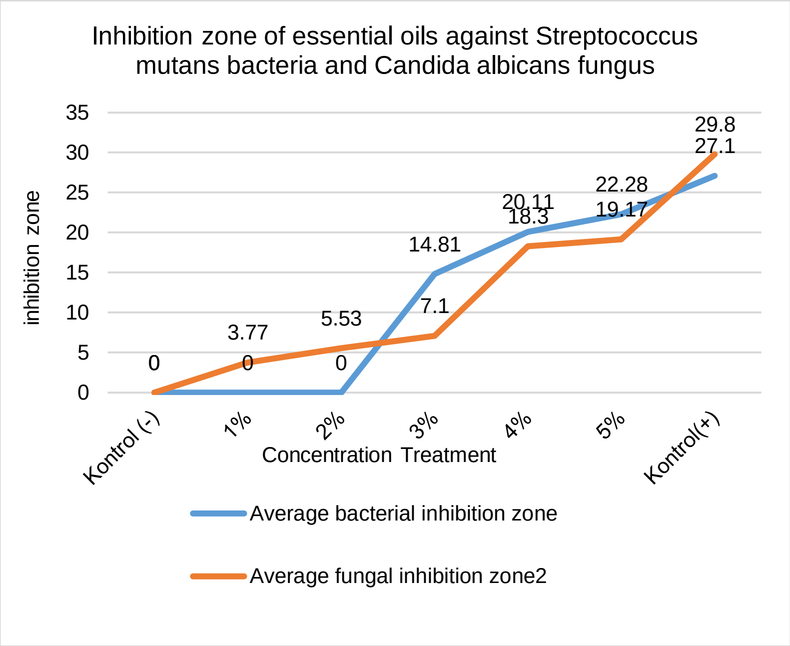

Figure 1. Inhibition zone graph of essential oils on *Streptococcus mutans* bacteria and *Candida albicans fungus*

The results of the calculation of the inhibition zone (clear) carried out on the essential oil of musk orange (*Citrus microcarpa* bunge) against the growth of *Streptoccocus mutans* bacteria have concentrations including 1%, 2%, 3%, 4%, 5%, negative control and positive control. The test results of 1% and 2% did not have an inhibition zone (0 mm), while concentrations of 3%, 4% and 5% produced diameters of 14.81 mm, 20.11 mm and 22.28 mm. The positive control using chloramphenicol produced an inhibition zone of 27.1 mm, while the negative control using (DMSO) produced an inhibition zone of 0 mm and there was no antibacterial activity. This shows that the effectiveness of essential oils increases with increasing concentration.

Based on Pharmacopoeia IV (1995), if an inhibition zone of 14 mm-16.5 mm is formed, it is said to be effective. Therefore, the essential oil of musk orange (*Citrus microcarpa* bunge) against the growth of Streptococcus mutans bacteria showed effective power at a concentration of 3%, which had an average of 14.81 mm, but at concentrations of 4% and 5% produced a very strong inhibition zone. These results support the potential of musk orange essential oil as a natural antibacterial, especially at medium to high concentrations. Further analysis was carried out using ANOVA which was already effective and Duncan's Test to see significant differences between treatments.

The Duncan test results showed that the DMSO negative control results were significantly different from the treatments at concentrations of 1% to 5%. This indicates that the DMSO solution does not have antibacterial properties. The highest average values were found in the 4% and 5% concentration treatments. At a concentration of 5%, it was not significantly different from 4% but was significantly different from concentrations of 1%, 2%, and 3%. This was influenced by the difference in concentration treatment, due to the addition of the concentration of musk orange essential oil (*Citrus microcarpa* bunge).

The greater the concentration of essential oil used, the greater the data obtained. While in this positive control, the antibiotic chloramphenicol was used which had the highest average value so that the positive control was significantly different from all concentration treatments of 1%, 2%, 3%, 4%, and 5% as seen from the Duncan test symbol/notation. According to Helmidanora et al. (2023) Chloramphenicol is a broad-spectrum antibiotic that has bacteriostatic properties by inhibiting protein synthesis in peptidyl transferase activity, this antibiotic is used for bacterial infections, chloramphenicol is also lipophilic.

Antifungal Activity Test of Musk Orange Essential Oil (*Citrus microcarpa* bunge) against *Candida albicans Fungus*

The antifungal activity test of musk orange (*Citrus mi*crocarpa bunge) essential oil was carried out using the disc diffusion method. Based on the results of the antifungal activity test of musk orange essential oil (*Citrus microcarpa* bunge) against the growth of *Candida albicans fungus*, it shows that at concentrations of 1% to 5% it produces an inhibition zone with a diameter that gets bigger with high concentrations, namely 3.77 mm, 5.53 mm, 7.1 mm, 18.3 mm and 19.17 mm. The highest inhibition zone was obtained at concentrations of 4% and 5% but the results of the inhibition zone did not exceed the positive control (Chloramphenicol) which had an inhibition zone of 29.8 mm and the negative control (DMSO) had no activity.

In the activity test results, musk orange essential oil (*Citrus microcarpa* bunge) has very different inhibition zone results. The difference in the diameter of this inhibition zone is influenced by differences in concentration, or the distance between the paper disc and the microbe, or when the paper disc is installed too tightly or incorrectly, which triggers differences in the inhibition zone results. However, based on the table above, the higher the concentration, the greater the inhibition zone results. Thus, musk orange essential oil has potential as a natural antifungal against Candida albicans, especially at concentrations of 4% and 5%. Which can potentially be used in the formulation of natural-based antifungal and antibacterial products as an alternative to synthetic compounds that have the risk of causing side effects or resistance.

The Duncan test results showed that the negative control (DMSO) concentration group produced the lowest value (0 mm), significantly different from the 1%, 5%, and positive control concentrations. This is because the DMSO solution lacks antifungal properties. Meanwhile, the 2% concentration was not significantly different from the 1% and 3% concentrations, but significantly different from the negative, 4%, 5%, and positive controls. Furthermore, the 4% and 5% concentrations were not significantly different but significantly different from the negative, 1%, 2%, 3%, and positive controls.

The difference in treatment is expected to increase the concentration of musk orange (*Citrus microcarpa* bunge) essential oil. The higher the concentration, the higher the average result obtained in this Duncan test. Furthermore, the positive control, chloramphenicol, was significantly different from the 1%, 5%, and 5% concentrations, as can be seen from the Duncan symbol/notation.

Test the Ability of Edible Film Preparations from Musk Orange Essential Oil (*Citrus microcarpa* bunge) in inhibiting the growth of *Streptococcus mutans* Bacteria

The results of clear zone growth at concentrations of 4% and 5% showed high inhibitory power against the growth of *Streptococcus mutans* bacteria with high average results of 21.58 mm and 22.36 mm. This indicates that the preparation has very strong antibacterial activity against *Streptococcus mutans* bacteria. As a comparison, the positive control using a xylitol-based product produced an inhibition zone of 28.25 which has very strong inhibitory power. According to Saputera et al., (2021), Xylitol has an effect in reducing the adhesion of *Streptococcus mutans* bacteria which is directly related to tooth decay that causes bad breath. Xylitol is able to influence bacterial metabolism by forming capsules in dental plaque that cause bad breath and tooth decay. Therefore, this study is in line because it produces high values in the positive control.

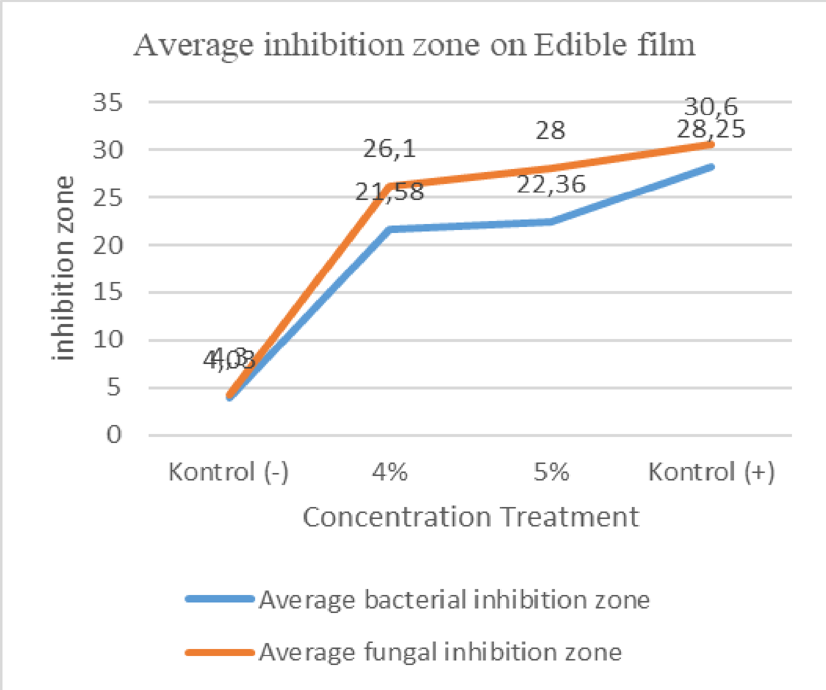

Figure 2. Inhibition zone graph of edible film preparation of essential oil on *Streptococcus mutans* bacteria and *Candida albicans* fungus

The negative control, using edible film without the addition of musk orange (*Citrus macrocarpa* bunge) essential oil, had an inhibition zone of 4.03 mm. This inhibition zone was due to the addition of other edible film ingredients, which can trigger the growth of the inhibition zone. Therefore, it can be concluded that good inhibition zone values are found at concentrations of 4% and 5%, but not exceeding the positive control concentration. The analysis results were effective, and further testing was conducted by Duncan.

The Duncan test results showed that the negative control concentration group (edible film preparation without the addition of musk orange essential oil) produced the lowest value (4.03 mm), significantly different from the 4% and 5% concentrations and the positive control. This is because the edible film preparation without the addition of musk orange essential oil produced a weak inhibition zone, but at other concentrations there were significant differences between one concentration and another. The 4% concentration was not significantly different from the 5% concentration, but there was a significant difference between the positive and negative controls.

Test the Ability of Edible Products Film Essential Oil Orange Musk (*Citrus microcarpa* bunge) in inhibiting the growth of *Candida albicans Fungus*

The test results showed that edible film with the addition of musk orange essential oil had a significant effect on the growth of *Candida albicans* fungus. The largest inhibition zone was obtained at a concentration of 5% with an average diameter of 28 μm, followed by a concentration of 4% (26.1 mm). Based on the category of antimicrobial activity (Ernawati & Jannah, 2021), an inhibition zone above 20 mm is very strong because the antimicrobial's ability to inhibit microorganisms is influenced by the type and concentration of a microbe, so that a large inhibition zone will be obtained. This is because the higher the concentration of the antimicrobial material, the more active substances it contains, thus increasing its effectiveness in inhibiting microbes.

However, the positive control had a higher value, at 30.6 mm. In this edible film preparation, it can be said that the 4% and 5% concentrations did not significantly outperform the positive control edible film packaging product (xyllitol gofress). Meanwhile, the negative control (edible film without essential oils) showed a weak inhibition zone resulting from the addition of edible film ingredients, proving that the antifungal activity indeed comes from essential oils. Essential oils are known to contain bioactive compounds with known antifungal activity, thus inhibiting the growth of Candida albicans. The ANOVA results were declared effective after the Duncan test.

The Duncan test results in this table also show that the xyilitol positive control group was significantly different from the 4% and 5% controls, as well as the negative control. Likewise, other concentrations, such as 4%, were significantly different from 5%, while the negative control was significantly different from the others. These results can be identified by the Duncan symbol/notation.

Organoleptic Test of Edible Film Preparations of Musk Orange Essential Oil (*Citrus microcarpa* bunge)

This organoleptic testing requires 20 panelists to see the quality of the edible film preparation, with characteristic tests including: Aroma, Color, Texture and Taste

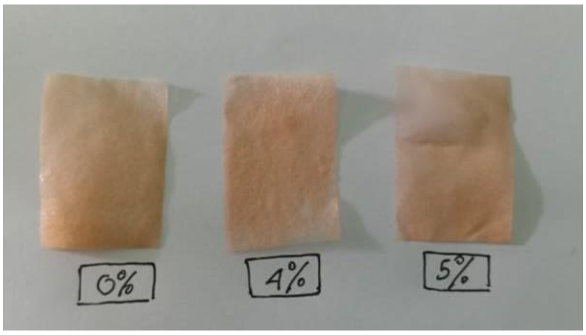

Figure 3. Edible preparation product of mustard orange essential oil (*Citrus microcarpa* Bunge) in inhibiting the growth of *Streptococcus mutans* bacteria and *Candida albicans fungus*

Aroma testing is a crucial parameter in organoleptic testing because it can influence consumer acceptance of the final product. Test results indicate that without the addition of essential oils, the product has a fresh, menthol-like aroma. This fresh aroma originates from edible film ingredients such as cornstarch or other additives, such as menthol, resulting in a neutral or refreshing characteristic, but does not yet exhibit the characteristic characteristics of musk orange. The addition of 4% essential oil produces a slightly musk orange aroma, while 5% produces a distinctive musk orange aroma, indicating that volatile compounds are beginning to evaporate (Fitri et al., 2024).

Adding the same orange essence to the color resulted in the same orange color. The consistent color results indicate that the addition of musk orange essential oil at concentrations of 4% and 5% did not significantly change the color of the edible film for panelists, making it an important parameter in the organoleptic assessment of edible film quality. While this difference in texture can be explained by factors influencing the texture of edible films, it actually stems from the addition of musk orange essential oil (*Citrus microcarpa* bunge), resulting in a less smooth and slippery film surface. Based on research by Bulin et al. (2023), essential oils are secondary plant metabolites containing nonpolar phenolic compounds at certain concentrations. Therefore, the active compounds in essential oils function as natural plasticizers or film-forming agents, helping to increase the homogeneity and flexibility of the film matrix. The higher the concentration of essential oil added, the smoother and more regular the film surface at each concentration.

Each concentration of edible film containing musk orange essential oil, whether at 4% or 5%, exhibits a characteristic "sweet" taste. This sweet consistency is due to the addition of sodium saccharin. According to Nuraenah et al., (2023), the addition of musk orange essential oil does not interfere with or mask the basic flavor of the edible film preparation, even at high concentrations. According to the Indonesian Minister of Health Regulation No. 208/MenKes/Per/IV/1985, the use of synthetic or artificial sweeteners is permitted and is listed in Sodium Saccharin. Saccharin is a white, odorless, water-soluble crystal with a sweet taste. Saccharin is also 200-700 times sweeter than sucrose. Therefore, the edible film formulation at every concentration has a sweet taste.

An irritation test was also conducted on the edible film product during the organoleptic test to determine the effects that occur in humans when the edible film experiment was carried out. It turned out that the results obtained from 20 panelists produced data that did not irritate. So it can be said that the edible film product from musk orange essential oil does not have an irritating effect and is safe for consumption because it goes through several other test stages.

According to previous research by Putra et al. (2017), which made edible film preparations from white turmeric essential oil only produced the best concentration at 2% (9mm) categorized as medium, and in previous research there have also been studies on the activity test of musk orange essential oil but did not test the formulation of edible film preparations from musk orange essential oil, so my research is the latest because there has been no research on the formulation of edible film preparations from musk orange essential oil (*Citrus microcarpa* bunge) in inhibiting the growth of streptococcus mutans bacteria and Candida albicans fungi and in this study the inhibition zone activity test was categorized as very strong because it had results like the graph above

Edible Preparation Thickness Test Mustard Orange Essential Oil Film (*Citrus microcarpa* bunge)

Edible film thickness testing is one of the parameters influencing the use of edible film in the formation of processed products to be packaged, and it is important to test it. The maximum thickness of edible film to meet the requirements of the Japanese Industrial Standard (JIS, 1975) is 0.25 mm. To measure the thickness of edible film, Krisbow digital calipers (Digital Vernier Calipers) can be used with an accuracy of 0.01 mm.

To see the actual comparison with Gofrees xylitol brand packaging products, the packaging product was weighed to within ±0.050 g. Then, each product concentration was weighed, yielding the same weight, at ±0.050 g. These edible products were weighed using an accurate analytical balance. Therefore, it can be concluded that the thickness of the edible film from musk orange (Citrus microcarpa bunge) meets the JIS thickness standard and also has the same weight as the circular packaging product, thus meeting the criteria for edible film characteristics.

Swelling test Preparation Edible Film Essential Oil Musk Orange (*Citrus microcarpa* bunge)

The swelling test on edible film was conducted to measure the extent to which the edible film absorbs water or other liquids. The relationship between variations in the concentration of edible preparations of musk orange essential oil on swelling. The swelling power test was conducted by inserting one sheet of film into a beaker and then dissolving it with 5 mL of distilled water. From the results of this study, the resulting time for the development of this product was approximately ± 3 minutes for each concentration of 0%, 4%, and 5%.

CONCLUSION

The characteristics of edible film preparations of musk orange (*Citrus macrocarpa* bunge) essential oil based on corn starch with the addition of musk orange essential oil showed an increase in antimicrobial ability along with the increase in the concentration of essential oil so that the resulting edible film has inhibitory power against microorganisms tested with concentration categories from weak to very strong depending on the concentration used and also has physical characteristics such as having a distinctive musk orange aroma, orange color, smooth texture and has a sweet taste at each concentration.

The effectiveness of edible film preparations of musk orange (*Citrus macrocarpa* bunge) essential oil in inhibiting the growth of Streptococcus mutans bacteria and Candida albicans fungus showed that the concentration of essential oil of 5% provided the highest inhibition zone against both microorganisms, which was 22.36 mm against Streptococcus mutans bacteria and 28 mm against Candida albicans fungus, this indicates that musk orange essential oil has the potential as a natural antimicrobial active substance in edible film preparations.

RECOMMENDATION

Future research on musk orange (*Citrus macrocarpa* bunge) essential oil–based edible films should focus on optimizing essential oil concentrations to determine the minimum inhibitory concentration and maximum effectiveness, testing against a broader range of microorganisms, and evaluating stability and shelf-life under various storage conditions. It is also recommended to explore synergistic combinations with other natural antimicrobials or antioxidants, assess practical applications on real food products, and conduct safety studies to ensure consumer health. In addition, studies on scalability, cost-effectiveness, and consumer acceptance are needed to support the commercial feasibility of these edible films.

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