Inhibitory Activity Test of Essential Oils Made from Pomelo, Sweet Orange, and Lime Fruit Peels Against Pathogenic Fungal Growth as an Antifungal in Animal Feed Ingredients

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Abstract. This study aims to determine the essential oils most effective in inhibiting the growth of pathogenic fungi (mold and yeast) in animal feed ingredients. Comparisons were made to the essential oils made from the peel of 3 types of citrus fruits: Pomelo, Sweet orange, and Lime. The research was conducted in 2016 at the Medicinal Plants Research Center and the Bogor Veterinary Research Center. Essential oils of all three types of orange peel were obtained by distilling in water and steam. The effectiveness of fungal growth inhibition was determined using the agar plate dilution method by using the Minimal Inhibitory Concentration (MIC) method. Furthermore, an analysis was carried out with a Gas Chromatography-Mass Spectrophotometer (GC-MS) to determine the content of essential oils in each orange peel. *Aspergillus spp., Fusarium spp., and Candida spp.* were selected as representatives of the fungal pathogen test. It was revealed that the content of essential oils in each orange peel used differed. It was concluded the essential oil from the Lime peel had in its composition the most excellent antifungal properties compared to the Sweet orange and Pomelo.

Keywords: MIC · Orange Peels · Essential Oil · Fungi

1 Introduction

Essential oil is a product of steam distillation from certain plant parts. This oil can contain tens or hundreds of volatile and non-volatile mixtures. This part is the cause of the characteristic aroma and taste. An important part of essential oil production is the refining process. Based on the boiling point, this process separates components into liquids or solids from two or more mixtures. At the beginning of the distillation process, the elements with lower boiling points will be distilled first, followed by those with higher boiling points. The yield and quality of refined essential oils depend on the quality of the fine raw material and the treatment before and during the refining process. The composition of the ingredients contained in essential oils can only be identified by

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conducting an analysis, which generally uses gas chromatography. This tool can separate volatile materials so they can be quantified.

Essential oils are used as a mixture of properties in raw materials in the cosmetics, soap, and detergent industries, pharmaceuticals, food and beverage products, and other products for the health and productivity of livestock. In the livestock sector, essential oils for broiler chickens, such as carvacrol in female broilers, have also been developed to reduce Feed Conversion Ratio (FCR) and lower plasma triglycerides [1, 2]. The provision of flavonoids, hesperetin, and naringenin extracted from the orange peel as supplementary feed has antioxidant properties and improves the performance of laying hens while producing eggs with lower cholesterol content [3]. Adding citrus bergamot essential oil (*Citrus bergamia*) positively affects the production, quality, fatty acid composition, and egg yolk of laying hens [4]. The essential oil mixture significantly affects broiler chickens' growth and organ weight [5]. In addition, essential oils as antifungals in the constituent ingredients of feed are new because these essential oils are also helpful for the health and improvement of poultry and ruminant productivity. This study aims to study the inhibitory power of essential oils from the peels of Pomelo, sweet orange, and Lime, which are the most effective for inhibiting the growth of fungi (mold and yeast). Furthermore, its ability as an antifungal in the feed had been tested as in the previous treatment [6–8].

2 Materials and Methods

2.1 Preparation of Test Materials

The research was conducted at the Research Institute for Spices and Medicinal Plants and the Center for Veterinary Research Bogor for over a year, from 2016 to 2017.

The determination of citrus fruit was taken from the peel to make essential oil. The types of citrus fruits were the Pomelo (*Citrus maxima*), Sweet orange (*Citrus aurantium* L), and Lime (*Citrus aurantifolia*). Then comes the distillation of the essential oil from the peels of the oranges. Meanwhile, molds and essential oil were manufactured from citrus fruit peel, and the essential oil was extracted by steam distillation and water. The peel of fresh citrus fruit was separated from the fruit until only the skin was obtained. The skin was cleaned and wilted for 24 h. The wilted skin was weighed as much as 25 kg; then, the distillation process was carried out. The distillate was filled with approximately 10 L of water, and then 25 kg of orange peel was put into the distillation area. The distillation process was carried out at a temperature of 110–115 °C for 6 h. The essential oil obtained was accommodated in a condenser and removed through a distillation faucet. The essential oil obtained was then added with anhydrous Sodium Sulfate, and then the filtrate was separated by filtering it with filter paper to get the water-free essential oil. The oil was stored in a tightly-closed container [9, 10].

2.2 Examination of Essential Oil Content Using GC-MS

Identifying the components of essential oil preparation was carried out using GC-MS equipment. The compound's structure was determined using a known standard by matching the fragmentation of the mixture in the library database. Each peak that appears in the chromatogram has different retention [11, 12].

2.3 Essential Oil Dilution

A total of 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1 ml of essential oils were put into test tubes, each containing 9.8; 9.6 ml; 9.4 ml; 9.2 ml; and 9 ml of sterile distilled water. After that, the essential oil solution was homogenized with a vortex mixer to obtain the essential oil dilution with concentrations of 2%, 4%, 6%, 8%, and 10%.

2.4 Preparation of the Tested Isolates

The fungal isolates to be tested were *Aspergillus flavus, A. fumigatus, A. niger, A. ochraceus, Candida albicans, C. krusei, C. parapsilopsis, C. tropicalis, Fusarium moniliforme*, *F. oxysporum, and Penicillium* sp, from the IRCV culture from the existing culture, were rejuvenated on Sabouraud Dextrose Agar (SDA) media. Colonies that grew from each of the two colonies that grew from each fungal cultures were taken as much as two thin oses and inoculated into 9 ml of sterile distilled water in a test tube and then shaken using a vortex to make it homogeneous. 1 ml of suspension was transferred to a pipette and then moved into a tube. The second, containing 9 ml of sterile distilled water, was then homogenized using a vortex. The repetition was repeated so that there were three tubes of suspension inoculum. Furthermore, colonies were counted using a hemocytometer using 0.1 ml of suspension inoculum from the 3rd test tube, pipetted, and then placed on the hemocytometer. Colonies were observed and counted using a microscope to obtain each fungal suspension containing 105 CFU/ml. This suspension will be used as a test material [13].

2.5 Preparation of Test Materials

To determine the effectiveness of essential oils from orange peel, essential oils containing 100% sterile distilled water and Tween 80 were used as emulsifiers. Essential oil concentrations range from 0% to 10%. To make 10 ml, each concentration was mixed with sterile distilled water as a solvent and Tween 80 as an emulsifier.

2.6 Inhibitory Test Determination of the Diameter of the Inhibitory Power Against Fungi

A total of 14 ml of SDA was poured into a Petri dish and allowed to solidify. As much as 1 ml of mushroom inoculum suspension was pipetted and poured into SDA, which had hardened in a petri dish, and then leveled with a spreader glass. As much as 5 ml of SDA with a temperature of 10–100 °C was added back into the petri dish containing the inoculum, leveled by shaking, and allowed to harden. The media that has been solidified is then perforated using the base of the Pasteur pipette into as many as five holes for each petri dish, with a diameter of 0.5 cm, so that each hole contains 0.1 ml of essential oil. Then it was incubated for three days at a temperature of 15–20 °C. Observations were made by calculating the clear zone that arose around the mushroom pit area. Observations were made from the 3rd to the 7th day.

Table 1. The content of antifungal essential oil components in the peel of Pomelo, Sweet orange, and Lime

Orange Species	Content of antifungal essential oil (%)
Pomelo	Cinnamaldehyde 10,84%
Sweet orange	α Pinen 1,00%; Sabinen 0,50%
Lime	α–Sitral 8,73%; α Pinen 1,25%; Sabinen 1,81%; Limonene 33,3%

2.7 Determination of the Minimum Inhibitory Concentration (MIC) of Fungi

The test was carried out using the plate dilution method by pouring 1 ml of each variation of the concentration of essential oil from citrus fruit peels into a petri dish. After that, as much as 3 ml of SDA was poured into a petri dish and then leveled by shaking to form a number 8 until homogeneous. Furthermore, 1 ml of mushroom suspension was added to a petri dish containing SDA and essential oil and leveled again. After that, it was incubated for three days at a temperature of 15–20 °C. This was repeated three times. Observations were made by monitoring at what concentration the yeast or mold began to grow and stopped growing. Observations were also made from the 3rd to the 7th day.

3 Results and Discussion

Essential oils, including those derived from citrus peels, can be used as antimicrobials, including antifungals [14–22]. Table 1 demonstrates that Lime peel essential oil concentration was more diverse and higher in % than Pomelo and Sweet orange peels. As a result, the antifungal effect was observed at the most significant percent against pathogenic fungus growth (Table 2).

Terpenoids are bioactive chemicals found in essential oils. Terpenoids have been found to block the manufacture of ergosterol, a key component of fungal cell membranes. The absence of ergosterol in fungal cell membranes damages the structure and function of cell membranes, resulting in fungal growth suppression.

Because the lipolytic part of terpenoids causes changes in membrane permeability and function in transferring vital molecules, metabolic imbalances can emerge, resulting in growth inhibition and cell death [23–25]. Essential oils contain hydrophobic components that can alter and impair membrane permeability, eventually leading to cell death. Furthermore, terpene-containing essential oil molecules can interfere with enzymes linked to fungal cell membranes, causing cell membrane function to be disrupted [26, 27].

The essential oils of Lime (*Citrus aurantifolia*) are categorized as terpenoids (α–Sitral 8.73%; pinene 1.25%; Sabinen 1.81%; limonene 33.3%). (Table 2). Based on these findings, α-citral is one of the essential volatile oil components as an antifungal [14, 28]. Citral is a potent allelopathic chemical with antifungal properties. Essential oils are comparable to azole antifungal drugs in that they interact with C-14 demethylase (cytochrome P-450 enzyme) to block lanosterol's demethylation into ergosterol, a necessary sterol for fungal membranes [29]. The cell membrane's permeability function will be harmed due to this inhibitory procedure.

Table 2. MIC of Essential Oils of Pomelo, Sweet orange, and Lime peel on agar media's growth of molds and yeasts.

No	Pathogernic Fungi	Essential oil from the peel of Fruits		
		Lime	Pomelo	Sweet orange
		MIC (%)	MIC (%)	MIC (%)
1	*Aspergillus flavus*	3,1	-	-
2	*A. fumigatus*	3,4	1,8	.
3	*0A.niger*	4,4	-	-
4	*A. ochraceus*	4,8	-	-
5	*Candida krusei*	5	-	-
6	*C.parapsilopsis*	3,3	-	-
7	*C.tropicalis*	7,8	-	-
8	*C. albicans*	-	2,1	Not Effective
8	*Fusarium moniliforme*	5,8	-	-
9	*F. oxysporum*	5,6	-	-
10	*Penicillium sp*	0,6	.	10

Limonene (26.04%), beta-citral (10.40%), beta-pinene (18.84%), α-citral (13.09%), and beta-phellandren make up the essential oil composition of Lime, according to the literature (6.29%) [30]. In addition to citral, limonene beta-pinene, and beta phellandren, the test results include cyclobutane and mycerene. This could be because the fruit is grown in several types. Citral's antifungal activities, however, remain prominent. Pomelo possesses only 10.84% antifungal Cinnamaldehyde on inspection, but pinene and limonene chemicals are not discovered, despite [31] claiming they should be.

According to the statistical calculations using the SAS program, it was found that the content of essential oils in each orange peel used was significantly different ($P < 0.05$), with the Lime essential oil having a greater MIC concentration than Pomelo and Sweet orange. Therefore, the Lime essential oil was the most effective in inhibiting fungal growth compared to the Pomelo and Sweet orange. The essential oil from the lime peel will be potential antifungal and can use the other place and substances. Furthermore, the essential oil from the Lime peel has been tested as an antifungal in feed, according to [6–8], with promising results [32].

4 Conclusions

The content of essential oils in each orange peel used was significantly different, the Lime essential oil having a greater MIC concentration than Pomelo and Sweet orange. It could be concluded that the Lime essential oil was the most effective in inhibiting fungal growth compared to the Pomelo and Sweet orange.

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