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Antioxidant and Inhibition of Essential Oil of Siam Citrus Peel (*Citrus Nobilis* L.) Kintamani Test Against *Candida albicans* hat Caused Candidiasis Fungal Infection

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Abstract: Siam citrus (Citrus nobilis L.) is one of the annual fruit crops found across Indonesia. Kintamani siam citruses are common in the Kintamani sub-district due to their high productivity and easy cultivation. However, it thas been discovered that siam citrus peel contains several benefits such as antioxidants and antifungals. Antioxidants can preserve food quality as well as health and beauty by inhibiting oxidation reactions such as binding free radicals and highly reactive molecules, thereby preventing cell damage. Candidiasis is an infection that affects several body tissues such as in oral cavity, skin, and can also attack the vagina. Candida albicans is the fungus that causes candidiasis. This study aimed to determine the ability of siam citrus peel essential oil activity at reducing free radicals and the growth of Candida albicans. Hydro steam distillation was used to extract the essential oil of the citrus. The components of the compounds contained in the essential oil of siam citrus peel were identified using Gas Chromatography-Mass Spectrophotometry, the antioxidant activity of essential oil in reducing free radicals was tested using the DPPH 0.1 mM or 40 ppm method, and the inhibition of siam citrus peel essential oil was tested by agar well diffusion method. The results showed very low antioxidant activity with an IC50 in the amount of 55475.63 ppm. However, essential oil of siam citrus peel showed very strong inhibition against C. albicans at a concentration of 1% providing an inhibitory diameter of 21 mm in agar medium.

Keywords: Antifungal; Antioxidant; *Candida albicans*; *Citrus nobilis* L; Essential oil

1. Introduction

Siam citrus (*Citrus nobilis* Linnaeus) is an annual fruit plant of the *Rutaceae* family. Siam citruses (*C. nobilis*) resemble tangerines at first glance, and the color of this citrus variety is generally green when cultivated in the lowland areas. At an altitude of about 950 m above sea level, such as in Punten-East Java, the skin color of siam citruses is evenly yellow when physiologically ripe. According to morphological diversity analiysis in the Balitjestro siam citrus collection, almost all accessions show similarities in leaf and fruit morphological characters. Through the analysis of RAPD molecular markers, the closeness of the genetic relationship of 16 collected citrus accessions is confirmed by 80-100%. Meanwhile with ISSR primers, the 16 collected citrus accessions show 74-92% closeness relationship to the tangerine (Agisimanto, 2020).

Indonesia has several commercial and superior citrus species, including tangerines, siam citruses, and large citruses (pamelo). Siam citrus is one of the citrus that is widely cultivated by farmers due to its high productivity and wide range adaptability from lowlands to highlands (KEMENTAN, 2021). Siam citruses are the most widely cultivated citrus by business actors, accounting for approximately 80% of citrus plantings in Indonesia (Balitbangtan, 2007).

Kintamani Siam citrus is a type of citrus cultivated in Kintamani District, Bangli Regency. Belancan Village is a farming village for citrus commodities with sweet and fresh flavors because it has soil, climate, altitude, and temperature conditions that are suitable for citrus plant cultivation. All of this time, people only consume the flesh of the siam citrus fruit. However, it thas been discovered that siam citrus peels have several health benefits, including anti-cholesterol, preventing heartburn, nourishing digestion, improvinge breathing, preventing cancer, relievinge bronchitis, curinge infections, flu, fever, and many more. Therefore, siam citruses are also included as herbal plants with high antioxidant activity (Samatha, 2012). This is due to the fact that siam citrus can be used as an essential oil. Essential oils are secondary metabolite compounds that volatile. Citrus peel essential oil contains bioactive compounds such as coumarin, flavonoids, carotene, terpene, limonene, etc. (Mondello, 2005). Some of these compounds have the ability to reduce free radicals (Juanda, 2017).

Furthermore, limonene, which accounts for approximately 70-92% of this dominant siam citrus, has activity in inhibiting the growth or killing the fungus by damaging its cell structure (Prabajati R, 2017). According to Chee HY (2009) limonene found in essential oils from his research, has a strong antifungal effect against *Trichopyton rubrum*. Other studies have also proven that the essential oil extract of Semboro siam citrus peel from Jember can inhibit *Candida albicans* fungi (Rana *et al.*, 2021).

Based on its geographical location, Indonesia has a humid tropical climate (Satwiko, 2008). In tropical climates, most *Candida* infections are superficial, affecting the skin, oral cavity, and vagina (Setiati, 2014). As previously stated, the essential oils in citruses contain antioxidant and antifungal compounds. So, it is necessary to conduct research related to antioxidant activity and inhibition of essential oil of siam citrus peel (*Citrus nobilis* L.) Kintamani against *Candida albicans* candidiasis fungus. The purpose of this experiment is to determine the ability of siam citrus peel essential oil activity in reducing free radicals and its ability to inhibit the growth of *C. albicans*.

2. Methodology

Materials

The main ingredients in this research were siam citrus peel (*Citrus nobilis* L.) and *Candida albicans* stock culture. While other materials the support this research are DMSO (*Dimethyl Sulfoxied*), Ciprofloxacin Antibiotic 0.5%, NA (Nutrient Agar), NB (Nutrient Broth), DPPH, Methanol, Sterile Aquades, and Alcohol (70%).

Tools

The tools used were UV-Vis Spectrophotometer Shimadzu 1800, Autoclave, Oven, Measuring Glass, Erlenmeyer, Beaker Glass, Drop Pipette, Socorex, Vortex, Micropipette, Tip, Oil and Water Separator Flask, Glass Stopper, Distillation Pipe, Cimarec Stirrer, Stative and Clamps, Distillation Pipe, Wrapper, Reaction Tubes, Measuring Flask, Analytical Balance, Laminar Air Flow Cabinet (LAF), Cork borer, Surgical Knife, Ose Needle, Vaseline Lup, Electric Stove, Gas Chromatography-Mass Spectrometry (GC-MS) Set, Stationery, and Camera.

Methods

Essential oil distillation and antifungal testing was conducted at the Bali-BRIN "Eka Karya" Botanical Garden Plant Potential Laboratory. Citrus fruit samples were obtained from Madam Suka Farm, Belancan Village, Kintamani District, Bangli Regency. Antioxidant testing was conducted at the Genetic Resources and Molecular Biology Laboratory of Udayana University, while GC-MS testing was conducted at the Laboratory of the Police Criminal Investigation Branch Denpasar, from March to December 2022.

Preparation of Siam Citrus Peel Essential Oil

The process of making essential oil of siam citrus peel (*Citrus nobilis* L.) Kintamani begins with the preparation of citrus peel samples by weighing them. The hydro steam distillation method is then used for distillation. Distillation was performed 3 times using sample material from different citrus peel harvest time. Furthermore, in the first 30 minutes during distillation, water vapor droplets will appear and then transferred to the oil and water separation flask in order to separate the oil that mixed with water. The amount of oil produced is then determined using a micropipette during the oil separation process.

Yield is the ratio of the amount (quantity) of oil produced from plant extraction. The yield uses units of percent (%). The percentage of essential oil was calculated according to Wibawa (2019) using Equation (1).

$$\%\ Yield = \frac{Volume\ of\ destilled\ oil\ (ml)}{Mass\ of\ material\ that\ wa\ distilled\ (gr)}\ x\ 100 \quad \ldots\ldots(1)$$

Specific gravity is a material constant or determination that adjusts to a homogeneous solid, liquid, gaseous form (Abellio N, 2015). The principal method for determining the specific gravity of essential oils is done by using a 2 ml volumetric flask. This calculation is a basis that used as a standard because it is easy to purify (Voigt, 1994). The following is the calculation formula as shown in Equation (2).

$$Specific\ gravity = \frac{Essential\ oil\ weight - Volumetric\ flask\ weight}{2} \quad \ldots\ldots(2)$$

In the process, the volumetric flask is cleaned and dried, then weighed in an empty state. Then, weigh the volumetric flask with the essential oil filled, observe, and record the results.

Essential Oil Test Againt *Candida albicans*

In the implementation of this stage, it is necessary to make nutrient agar (NA) and nutrient broth (NB) media. Making NA requires 500 ml of distilled water with 14 grams of NA, then stirring until the froth disappears

and the NA solution appears clear. Likewise, for the process of making NB, 3.25 grams of NB and 250 ml of distilled water are required to make 10 ml of test solution. As much as 8 ml of NA will be used as a sloping medium.

Furthermore, the preparation stage of *C. albicans* obtained from stock culture. Reculture is required by performing microbial culture, which aims to rejuvenate the colonies of the stock culture colonies. *C. albicans* is struck zig-zag on slanted NA media using a round tip ose in a laminnar, then incubated for 24 hours at 37^0C. There will be soft colonies with yeast-like aroma that are, white, and smooth. Then *C. albicans* can already be suspended in 10 ml of NB solution and left for 24 hours at the same temperature to see if the culture process is successfull, which will appear cloudy.

In the control solution preparation stage, a positive control antibiotic ciprofloxacin was used, this antibiotic has a mechanism of action in inhibiting nucleic acid synthesis which inhibits DNA gyrase replication (topoisomerase II) with quinolone content that can bind to fungi (Joseph Meletiasid, 2008 and Mycek, 2001). Meanwhile, DMSO is used as a negative control. The process of making a K^+ solution of 0.5% ciprofloxacin antibiotic is 50 ml of sterile distilled water plus 0.5 grams of ciprofloxacin combined, then taking as much as 1 ml of this solution adding 10 ml sterile distilled water. While in the of 10% K^- DMSO solution, 1 ml of DMSO is added to sterile distilled water to get 10 ml of ready-to-use solution.

The preparation stage of the antifungal test uses essential oil of siam citrus peel by making 10 derivatives ranging from 1% to 0.001953125%. During process, an initial concentration of 25% essential oil is made, requiring 250 µl of oil plus 750 µl of DMSO. Furthermore, using the solution dilution equation formula with the information of the initial volume of solution (V_1), final volume of solution (V_2), initial concentration of solution (M_1), and final concentration of solution (M_2) as shown in Equation (3).

$$V_1 . M_1 = V_2 . M_2 \quad (3)$$

Antifungal testing was performed at 1%; 0.5%; 0.25%; 0.125%; 0.0625%; 0.03125%; 0.015625%; 0.0078125%; 0.00390625%; and 0.001953125% concentratons of siam citrus peel essential oil as K^+ and 0.5% concentration of ciprofloxacin which was also treated as K^+. While K^- from 10% concentration DMSO solution. A total of 6 ml of NA was poured into an autoclaved glass petridish, then waited until it was solid. Furthermore, NB containing *C. albicans* which has been in suspension for 24 hours can be used, NB is vortexed first before testing to eliminate the homogeneity of the microbial solution. The NB is then applied with a sterile cuttonbud made of cotton wrapped around a toothpick and sterilized in an autoclave.

Diffusion wells were made with a cork borer in NA media smeared with NB. Then, using a micropipette, insert 8 µl of each control solution into the wells. Next, incubate in a 37^0C oven for 24 hours. If there is a clear area on the surface of the jelly media, it indicates that the inhibition of microorganism growth by antimicrobial agents occurs (Pratiwi, 2008).

Antioxidant Activity Test Using DPPH Method

This method is frequently used to test compounds that act as free radical scavengers or hydrogen donors and evaluate antioxidant activity. The DPPH method can be applied to both solids and liquid samples (Prakash, Rigelhof, and Miller, 2001). The testing stage employs a 0.1 mM or 40 ppm DPPH free radical compound which is made by putting 1 mg of DPPH into a 50 ml volumetric flask and diluting with methanol until the limit mark, resulting in a dilution of the control DPPH solution (blanko). Then test the antioxidant activity by dissolving the

essential oil sample in methanol solvent, which is then vortexed and incubated for 30 minutes. Enter the cuvette to be measured with a UV-Vis Spectrophotometer at wavelength of 517 nm.

According to Molyneux (2001), the attenuation/inhibition (Q) is sought using the sample absorbance's simplest approximation formula, as shown in Equation (4)

$$Q = \frac{Absorbance\ blanko - Absorbance\ sample}{Absorbance\ blanko}\ x\ 100\% \quad \text{.....(4)}$$

Data Analyses

Analysis of Oil Chemical Compounds Using GC-MS

The essential oil of siam citrus peel is analyzed to identify the constituent components present. Separated compounds from Gas Chromatography on the column will flow into Mass Spectrometry and be identified based on molecular weight. The GC-MS conditions used are Agilent Technologies 7890B Gas Chromatography System and 5977B Mass Selective Detector. Pressure 25,331 psi within 52 sec chromatograph initial temperature 70^0C to temperature 290^0C and has a total flow of 290 ml/minute, the oil sample was placed on Agilent 5190-2295: 870 µl. With the same pressure setting the MSD agilent 190915-433UI: HP5MS will have an average speed of 62.662 cm/sec and then look at the results of the identification of volatile components with their fragmentation patterns.

Gas chromatography has a wide range of applications, it can be used to separate and analyze mixtures of several components. The results of gas chromatography show the chromatogram of the distilled essential oil of siam citrus peel. Futhermore, each peak in the chromatogram is identified by matching the MS spectrum of each peak with the Wiley data base to determine the type of compound (Hartono, 2017). Gas chromatography is able to read compounds with the lowest concentration, allowing secondary metabolites in plants to be identified with the results of chromatograms and mass spectra. (Al-Rubaye *et al.*, 2017).

Data Analysis of Antioxidant Activity

Analysis of antioxidant testing DPPH method using this data processing technique compares the concentration with the % antioxidant activity value of each sample dilution concentration in a regression graph. The IC_{50} value is then subtended by the y value, will yielding the x value as the IC_{50} value.

Analysis of Essential Oil Inhibition Against *Candida albicans*

The collected data were edited and tabulated to determine the average inhibition zone (mm) of Kintamani siam citrus peel essential oil against *C. albicans* growth.

3. Results and Discussion

Yield and specific gravity of essential oil

Essential oil from Kintamani siam citrus peel is recorded by weighing the initial weight of citruses, citrus peel, until it is capable to produce essential oil, shown in Table 1. This activity aims to determine the capacity of gram fruit in producing oil to obtain yield and specific gravity of oil. Based on Table 1. the results of Kintamani siam citrus peel distillation have a high yield of 0.211%. Oil yield can be influenced by pre and post harvest factors. Harvest time is one of the harvest factors that influences oil yield. Futhermore, post harvest factors that influence oil yield include of distilling materials and distillation methods (Prianto, 2013).

Table 1. Results of distillation of Kintamani siam citrus peel

Distillation	Citrus Fruit (Gr)	Peel Weight (Gr)	Oil Weight (Gr)	Oil Volume (Ml)	Yield (%)	Specific Grafity (Nm^3)
I	5.666	1.352	14,733	2,86	0,211	0,785
II	10.414	2.523	13,164	4,85	0,192	0,001
III	6.444	2.170	14,714	1,85	0,085	0,776

Note: Distillation I (April 6); Distillation II (April 27); Distillation III (May 19)

Harvesting time for distillation sample I was conducted on April 6 and distillation II on April 27, and distillation III on May 19. Reported from Indonesian Agency for Meteorological, Climatological and Geophysics (Badan Meteorologi, Klimatologi, dan Geofisika or simply BMKG) the dry season begins in April until its peak in August. The North Bangli region, especially Kintamani District, has geographical conditions in the highlands, which affects the climate and the rotation or meeting of air currents caused by the mountains, resulting in relatively high rainfall in this area (Warsudi, 2022). Harvesting for distillation sample III (May 19) occured during a period of high intensity rainfall. Climatic conditions during harvesting samples are one of the factors that influence the yield of essential oil of Kintamani siam citrus peel.

Harvesting when the intensity of rainfall is relatively high usually results in a lower yield of essential oil than when it is dry (Setyawan, 2002). According to Armando (2009) the higher the yield the lower the quality of the oil. From the statements, it is known that essential oil distillation I produces low quality oil, compared to distillation III because the yield value is higher. The difference in essential oil yield is also thought to be due to the location of growth and the presence of other plants nearby that affect biosynthesis and macro elements for a plant (Wibawa, IPAH, 2019; Wibawa, IPAH, Venna, & Wawan, 2019).

According to Sagel (1993), density is the measurement of an object's mass per unit volume. The higher the density of an object, the greater the volume mass or weight of the object. This statement is in accordance with the research findings shown in Table 1. the weight of citrus peel essential oil distillation I of 14.733 grams has a density of 0.785 Nm^3. The density of distillation I is not significantly different from the results of distillation III, which is the density of 0.776 Nm^3 of oil weight of 14.714 grams. In contrast to distillations I and III, distillation II for oil weight of 13.164 grams only has a density of 0.001 Nm^3. Specific gravity is often related to the weight fraction of the components contained in the oil. The greater the weight fraction contained in the oil, the greater the specific gravity value of the oil (Lutony & Rahmawati, 1994). In Guanther (1990) research, the length of distillation can influence the evaporation of high boiling fractions. The longer the distillation, the more evaporation of the high-boiling fraction will occur.

Analysis of Essential Oil Compounds

Essential oil of Kintamani siam citrus peel was analyzed on the compounds contained using Gas Chromatography-Mass Spectrophotometry (GC-MS), the results of the separation of essential oil components can be seen in Figure 1.

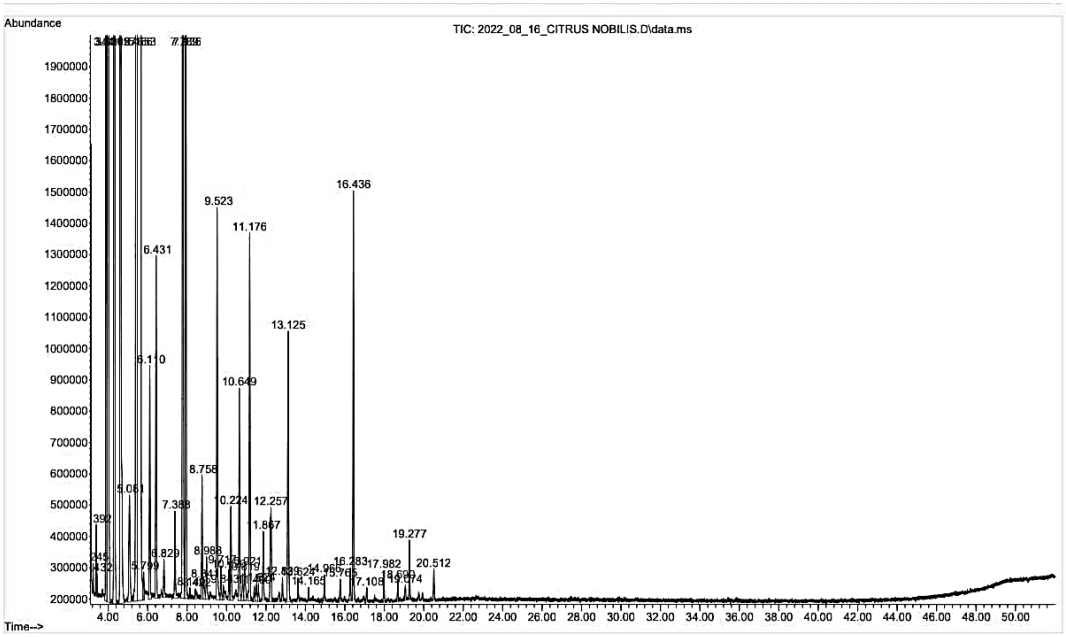

Figure 1. Essential oil components of Kintamani siam citrus peel

The chromatogram data of the essential oil components of siam citrus peel produced from steam and water distillation shows quantitative data indicating the relative abundance of many compounds in the sample. From the results will be seen the retention time of each separation of the compound components of citrus peel essential oil, area, height of each chromatogram peak, relative concentration of each compound component with mass spectra data of each component including the name of the compound component, and the use of existing compounds to identify compounds. The main compunds contained in the of essential oil of siam citrus peel (*Citrus nobilis* L.) Kintamani are listed in Table 2.

Table 2. Compound content of essential oil of siam citrus peel (*Citrus nobilis* L.) Kintamani

No.	Compound Name	Compound Use	MW (gr mol^{-1})	AUC (%)	Ret. Time
1	Sabinene	Coronavirus Study (Asma Baiq., 2022)	136.23	2.56	3.901
2	beta-Pinene	Resin (ACGIH, 2020)	136.23	9.12	3.991
3	beta-Myrcene	Aromatics (The Chemical and Products Database, 2018)	136.23	4.04	4.309
4	Octanal	Aromatics (Larranaga, 2016)	128.21	2.96	4.627

No.	Compound Name	Compound Use	MW (gr mol^{-1})	AUC (%)	Ret. Time
5	Limonene	Flavoring, Aromatics, Insecticidal, insect repellant (PubChem, 2004)	136.23	15.14	5.466
6	D-Limonene	Flavoring, Aromatics, Insecticidal, insect repellant (PubChem, 2004)	136.23	57.45	5.653
7	Linalool	Aromatics (Merck Index-O'Neil MJ, (2013) and Hawley-Lewis RJ., (2007))	154.25	2.96	7.789
8	Linalyl formate	Falvoring (PubChem, 2005)	182.26	2.96	7.789
9	Nonanal	Aromatics (Hawley-Lewis RJ., 2007)	142.24	1.25	7.936

Information: AUC (Area Under Curve) = Abundance of detected compounds; MW (Molecular Weight) = Molecular mass in units of atomic mass; Ret. Time (Retention time) = Detection time of the compound

The essential oil fraction of siam citrus peel has a high molecular mass value. Thus, the greater the molecular mass, the higher the boiling point of the compound (Fessenden & Fessenden, 2017). The components of essential oil compounds are dominated by D-Limonene at 57.45%, followed by Limonene (15.14%), beta-Pinene (9.12%), beta-Myrcene (4.04%), Octanal (2.96%), Linaloll (2.96%), and Linalyl formate (2,96%), Sabinene (2.56%), and Nonanal (1.25%). These compounds are grouped into terpenoid compounds.

Antioxidant Activity Test Analysis

The antioxidant activity test using the DDPH method was analyzed using a Shimadzu 1800 UV-Vis Spectrophotometer on the first distillation sample (April 6) as follows,

Table 3. Absorbance results of essential oil of siam citrus peel 5 ml dilution

Sample Concentration (µl)	Absorbance	IC (%)
200	0,4441	9,94
400	0,4294	12,92
600	0,4127	16,31
800	0,3917	20,56
1000	0,3699	24,98

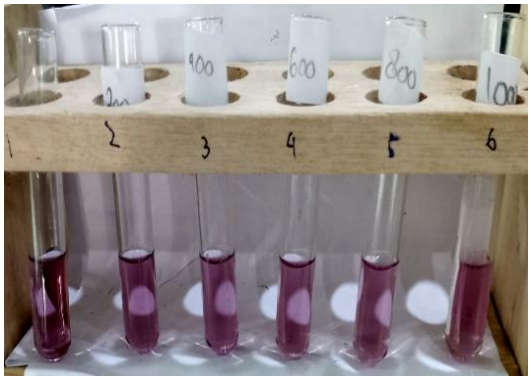

Figure 2. Color comparison of blanko with 5 ml dilution sample

At a dilution of 0.12 g 5 ml^{-1} with an initial sample concentration of 25,058 ppm in spectrophotometric results. The absorbance is directly proportional to the concentration of the substance. The higher the concentration, the higher its absorbance. This is because the proportion of light that gets absorbed is affected by the number of molecules that it interacts with presented in Table 3. Antioxidant testing with a concentration of 200-1000 µl incubated 30 minutes produces in the color display shown in Figure 2.

Table 4. Absorbance results of siam citrus peel essential oil pure sample

Sample Concentration (µl)	Absorbance	IC (%)
0	0,4931	0
100	0,2667	45,9136
200	0,1745	64,6116
300	0,1103	77,6313

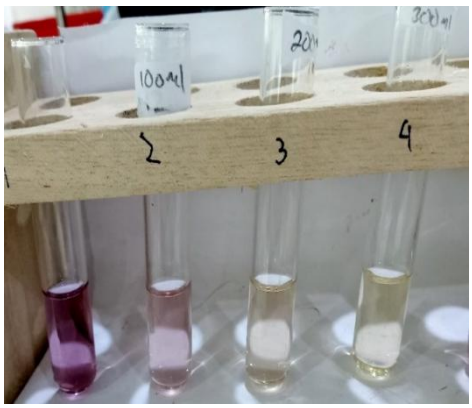

Figure 3. Color comparison of blanko with pure sample

Pure essential oil sample 0 µl 1 ml^{-1} without dilution of methanol solvent has a significant correlation to the suppression of DPPH free radicals as shown in Figure 3, which shown a color shift from purple to yellow. This is happened because the pure essential oil component contained in antioxidant testing has a terpenoid group bioactive compound component in the form of β-Myrcene compounds (4.04%). Mycrene is a compound with

strong antioxidant effects that can protect againt oxidative damage (Putra, 2020). Although the results of the 0 µl 1 ml^{-1} sample showed a clear color change in the test, the 0.12 g 5 ml^{-1} dilution treatment did not experience a color change. As the performance of chromophore and auxochrome groups on DPPH free radicals when giving maximum absorbance at wavelength (λ) 517 nm, which produces purple color. The color of DPPH will change from purple to yellow with the addition of antioxidants from siam citrus peel essential oil when a single electron on DPPH pairs with hydrogen from the antioxidant. However, dilution with 5 ml did not give such reaction. Therefore, a linear regression graph antioxidant analysis was conducted to determine 50% free radical silencing. This was completed by substituting the y value to get the x value as the IC_{50} value.

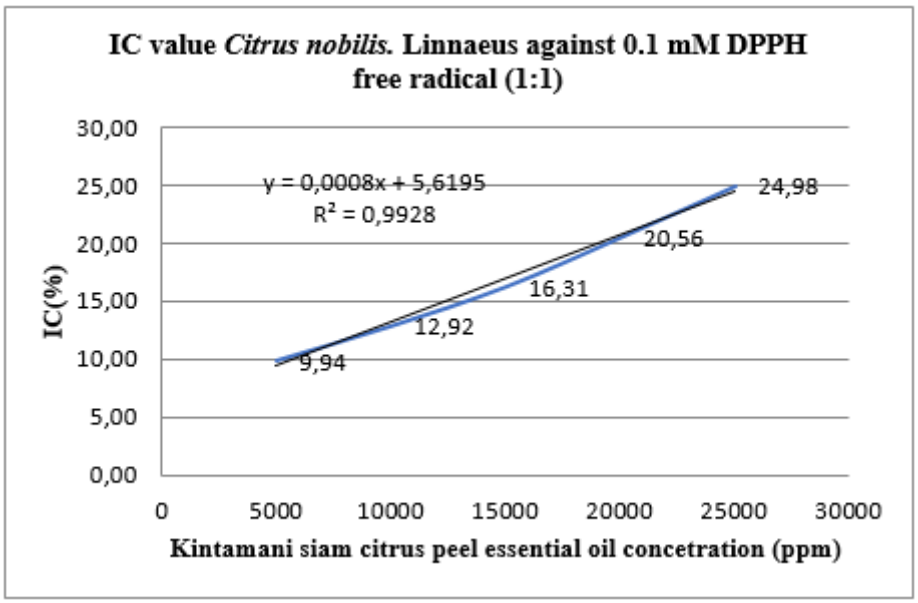

Figure 4. Regression absorbance graph of siam citrus peel essential oil

Based on Figure 4. the IC_{50} value of antioxidant testing against 0.1 mM DPPH free radicals for samples of essential oil of siam citrus peel has very weak category results. According to Zuhra *et al.*, (2008), by knowing the intensity of checking with a linear regression equation, the IC_{50} value of more than 500 ppm is a very weak antioxidant category. So that the essential oil of Kintamani siam citrus peel is categorized as very weak because it requires 55475.63 ppm to reduce 50% of free radicals. The use of this oil sample is influenced by the state of the sample using the first distillation sample (April 6) which has a high yield condition with high density (homogeneous). However, high yield implies poor quality of essential, so dilution does not provide optimal free radical suppression results.

The activeness of the class of compounds that function as free antiradicals is determined by the presence of free -OH (*hydroxyl*) functional groups and carbon-carbon double bonds, such as flavone, flavanone, scualen, tocopherol, β-carotene, vitamin C, and others. The reaction that occurs is the formation of *diphenylpicrylhydrazine*, through antioxidants's to donate hydrogen (Yanuar, 2002).

Inhibition Analysis of Siam Citrus Peel Essential Oil

Negative control testing using DMSO (*Dimethyl Sulfoxied*), also positive control (ciprofloxacin antibiotic) to see the width of inhibition against *C. albicans* as shown in Table 5 and Figure 5. K^- (DMSO) does not provide inhibition against *C. albicans* as in K^+ with a width of inhibition (LDH) of 29 mm which presented in

Table 5. This proves that the solvent used in creating the concentration derivative solution of Kintamani siam citrus peel essential oil used has no antifungal effect.

Table 5. Width of Inhibition (LDH) of control solution against *C. albicans*

Control	Concentrations (%)	Average (mm)
DMSO	10	0
Ciprofloxacin	0,5	29

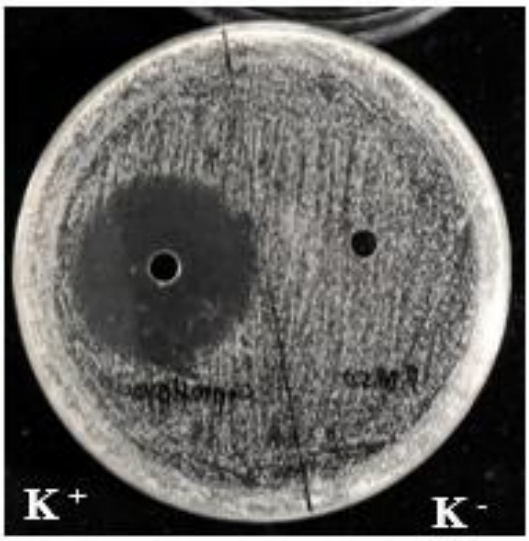

Figure 5. LDH assay results of positive control and negative control solutions

Essential oil testing to find the inhibition zone of *C. albicans* using a concentration of 1%; 0.5%; 0.25%; 0.125%; 0.0625%; 0.03125%; 0.015625%; 0.0078125%; 0.00390625%; and 0.001953125% are presented in Table 6. Based on the average diameter of the inhibition test of siam citrus peel essential oil against *C. albicans* in Table 6. it was known that the widest inhibition zone was at a concentration of 1% with a 21 mm wide inhibition zone, whereas at a concentration of 0.001953125%, it was only able to inhibit *C. albicans* as 5.5 mm wide.

According to Nopiyanti *et al.*, (2016), the diameter of the inhibition zone is categorized as very strong if ≥ 20 mm, strong 10-20 mm, and moderate if it is 5-10 mm, and weak if it is ≤ 5 mm. The inhibition zone given by essential oil of siam citrus peel is categorized as very strong (21 mm), strong (11 mm; 10.5 mm; and 10 mm), and moderate (9.5 mm; 9 mm; 8.5 mm; 8 mm; 7 mm; 5.5 mm). Therefore, Kintamani siam citrus peel essential oil is categorized as a strong oil, due to its ability to inhibit the growth of *C. albicans* at the smallest concentration (0.001953125%), which is categorized as having a moderate diameter. Figure 6. shows inhibition of Kintamani siam citrus peel essential oil against Candida albicans

Essential oil of Kintamani siam citrus peel can be used as an antifungal as shown in Figure 6 because of its ability to inhibit the growth of *C. albicans*. The content of D-Limonene compounds (57.45%) and Linalool (2.96%) is the main compound component of Kintamani siam citrus peel essential oil which affects dermatitis symptoms (Fatma Yasmin Mahdani *et al.*, 2020). This terpenoid group compound has a strong effect on *C. albicans* by inhibiting the synthesis of its growth. In terms of morphology, this compound alters the pectin structure of the *C. albicans* cell wall, disrupting its ability to adhere to the oral cavity epithelium. In addition to affecting cell structure, Limonene is also able to inhibit cell development, and disrupt cell respiration. Meanwhile, the active

compound Linalool can inhibit the production of enzymes that protect cell membranes (Fatma Yasmin Mahdani *et al.*, 2020).

Table 6. LDH results of Kintamani siam citrus peel essential oil against *C. albicans*

Concentration (%)	Average (mm)	Category
1	21	Very Strong
0.5	11	Strong
0.25	10.5	Strong
0.125	10	Strong
0.0625	9.5	Moderate
0.03125	9	Moderate
0.015625	8.5	Moderate
0.0078125	8	Moderate
0.00390625	7	Moderate
0.001953125	5.5	Moderate

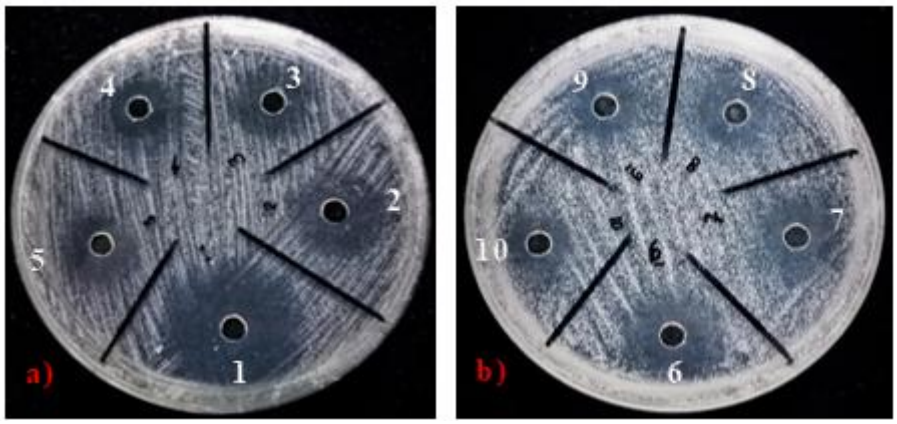

Figure 6. Inhibition of Kintamani siam citrus peel essential oil against *Candida albicans*

Descriptions:

a) Concentration: 1 (1%); 2 (0,5%); 3 (0,25%); 4 (0,125%); 5 (0,0625%)
b) Concentration: 6 (0,03125%); 7 (0,015625%); 8 (0,0078125%); 9 (0,00390625%); 10 (0,001953125%)

5. Conclusions

The compound components in the essential oil of Kintamani siam citrus peel were dominated by D-Limonene of 57.45%, followed by Limonene (15.14%), beta-Pinene (9.12%), beta-Myrcene (4.04%), Octanal

(2.96%), Linaloll (2.96%), Linalyl formate (2.96%), Sabinene (2.56%), and Nonanal (1.25%). The antioxidant activity of essential oils using 0.1 mM DPPH free radical was categorized as very weak with IC_{50} of 55475.63 ppm. The essential oil of Kintamani siam citrus peel had the potential to inhibit the growth of *Candida albicans* which was categorized as very strong with inhibitory diameter of 21 mm.

Author Contributions

If your research article has several authors (i.e., those who have contributed substantially to the work), you are recommended, but not required, to list the contributions of each author in the following statement: "Conceptualization, P.T.A and Y.H.; methodology, P.T.A and W.I.P.A.; software, P.T.A; validation, P.T.A, Y.H. and W.I.P.A.; formal analysis, P.T.A; investigation, P.T.A; resources, P.T.A; data curation, P.T.A; writing—original draft preparation, P.T.A; writing—review and editing, P.T.A; visualization, P.T.A; supervision, P.T.A and W.I.P.A.; project administration, P.T.A; funding acquisition, Y.H. All authors have read and agreed to the published version of the manuscript."

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Conflicts of Interest

The authors declare there no conflict of interest

References

Agisimanto, D. M. (2020). Differences in RAPD and ISSR primers in the identification of genetic kinship of Indonesian siam citruses (Citrus suhuniensis L. Tan). J. Hort., 17(2), 85754.

Armando, R. (2009). Producing 15 Quality Essential Oils. Bogor: Penebar Swadaya.

Asma Baiq, H. S. (2022). SARS-CoV-2 inhibitors from Nigella Sativa. Applied Biochemistry and Biotechnology, 194: 1051-1090.

Balitbangtan. (2007). Development and distribution of citrus crops in Indonesia. East Java: Innovative Technology.

Berman J, S. P. (2012). *Candida albicans*: A molecular resolution built on lessons from budding yeast. [Cited 2012 Jan 21], Available online at: http://www.nature.com/nrg/journal/v3/n12/box/nrg948_BX1.html.

Bouchassane, N., & Merad-Boussalah, N. (2021). Anti-Inflammatory, Antimicrobial and Insecticidal Properties of Daucus Gracilis Steinh Flowers Essential Oil. Antiinflam Antiallergy Agent Med Chem, 20(3): 264-270.

Chee HY, K. H. (2009). Antifungal Activity of Limonene against Tricophytonrubrum. J Microbiol, 37 (3): 243-246.

Fatma. Yasmin. Mahdani, D. A. (2020). Effectiveness of *Citrus Limon* Peel Essential Oil On *Candida albicans*: In Vitro Study. Biochem. Cell. Arch, 20 (1): 2957-2961.

Fessenden, R. J., & Fessenden, J. S. (2017). Organic Chemistry Volume I 3rd Edition. Jakarta: Erlangga.

Guanther, E. (1990). Essential Oils Volume I and IV A. Jakarta: Translator: University of Indonesia Press.

Hawley-Lewis RJ., H. (2007). Hawley's Condensed Chemical Dictionary 15 th. New York: John Wiley & Sons.

Wibawa, IPAH. V. S. (2019). A study of essential oil from an invasive Piper aduncum. Jurnal Biologi Udayana, 23 (2): 50-58.

Wibawa, IPAH. S. V. (2019). STUDY OF THE POTENTIAL OF OXYRIENTAL OIL OF Boenninghausenia albiflora (Hook.) Rchb. ex Meisn. IN EKA KARYA BOTANICAL GARDEN, BALI. Botanical Garden Bulletin, ISSN: 2460-1519. Vol. 22 (2): 73-82.

Juanda, D. B. (2017). total phenol content and antioxidant activity of fruit juice of five citrus species (Citrus sp.). Galenika Pharmacy Journal. 02(01): 37.

KEMENTAN. (2021). Siam citrus. In A. N. Kurniawan Budiarto, Innovative Technology of Healthy Citrus of the Archipelago (pp. 33-36). Bogor: PT Penerbit IPB Press.

Lee, R. X., & Reed, E. (2012). β-elemene effectively suppresses the growth and survival of both platinum-sensitive and resistant ovarian tumor cells. Anticancer, 32 (8): 3103-13.

Maharani, S. (2012). Effect of Siwak (Salvadora persica) Extract Solution at Various Concentrations on the Growth of Candida albicans. Yogyakarta: Faculty of Medicine, Diponegoro University.

Merck Index-O'Neil MJ, H. P. (2013). he Merck Index, An Encyclopedia of Chemicals, Drugs, and Biologicals, 15th. Cambridge: Uk: The Royal Society of Chemistry.

Nopiyanti, H., Agustirani, F., & Isnaini, M. (2016). Screening of Nypa Fructions as Antibacterial of Bacillus subtilis E. Coli and S. aureus. Journal Maspori, 8 (2) : 83-90.

Prabajati R, H. I. (2017). Effects of citrus limon essential oil (*Citrus.* Dental Journa, 50(1): 43-48.

Prianto, H. R. (2013). Isolation and Characterization of clove flower oil. Chemistry Student Journal, 269-275

PubChem. (2004). D-Limonene. PubChem. National Library of Medicine, Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/440917#section=Uses.

PubChem. (2004). Limonene. PubChem. National Library of Medicine, Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/22311#section=Uses.

PubChem. (2004). octan-1-ol. PubChem. National Library of Medicine, Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/957#section=Uses.

PubChem. (2005). Linalyl formate. PubChem. National Library of Medicine, Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/61040.

Rana, O. C., & Ayu Mashartini Prihanti. (2021). Inhibition of Essential Oil Extract of Siam Citrus Peel (Citrusnobilis L.) against the Growth of Candida albicans. e-Journal of Health Library, vol. 9 (3) : 166-170.

Sagel, R. K. (1993). Guidelines for Concrete Work. Jakarta: Erlangga.

Samatha, T. S. (2012). Quantification of Total Phenolic and Total Flavonoid Contentsin Extracts of Oroxylum indicum L.,Kurz. Asian Journal of Pharmaceutical and Clinical Research,, 5 : 178-180.

Setiati, S. d. (2014). Handbook of Internal Medicine. 2nd Edition. Jakarta Pusat: InternaPublising.

The Chemical and Products Database. (2018). a resource for exposure-relevant data on chemicals in consumer products. Scientific Data volume 5, p. 180125.

Voigt, R. (1994). Textbook of Pharmaceutical Technology V. Yogyakarta: UGM Press.

Warsudi, E. (2022). Forecast of the 2022 Dry Season in Indonesia. Available online at: https://www.bmkg.go.id/berita/?p=prakiraan-musim-kemarau-tahun-2022-di-indonesia&lang=ID&s=detil.

Yanuar, W. (2002). Antioxidant and Immunodulatory Activities of non-rice cereals. Malang: University of Muhammadiyah Malang.