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Effectiveness Test of Sunkist Orange Peel Ethanol Extract (Citrus Sinensis L. Osbeck) on the Growth of Candida Albicans Mushrooms as a Cause of Fluor Albus a Woman

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Abstract**—** *Around 90% of women in Indonesia have the potential to experience ALBUS FLUOR because Indonesia is a country with a tropical climate, the most common cause of vaginal discharge is Candida albicans fungal infection. Citrus fruit contains secondary metabolites such as flavonoids, alkaloids, coumarins, limonoids, keratonids, and essential oils which have pharmacological activities such as anti-bacterial, anti-fungal, antioxidant, anti-inflammatory, anticancer, heart-protecting, neuroprotective, and hepatoprotective. Ethanol of Sunkist orange peel against Candida albicans fungus as a cause of vaginal discharge at a concentration of 20%, 40%, 60%, 80%, and 100%. This study is an in vitro study used a laboratory experimental design (true experiment) with a post-test control group design only approach. Results: The diameter of the Inhibition Zone in giving Sunkist orange peel extract with a concentration of 20% was 9 mm, at a concentration of 40% was 12 mm, at a concentration of 60% was 13 mm, at a concentration of 80% was 14 mm, and at a concentration of 100% was 13 mm. Conclusion: Sunkist orange peel extract has significant antifungal effectiveness against Candida albicans fungi and the higher the concentration of the extract given, the larger the diameter of the inhibition zone formed.*

Keywords**—** *Ethanol extract, antifungal, candida Albicans, vaginal discharge.*

I. INTRODUCTION

Candidiasis is one of the most common causes of fungal infection in humans. Diseases that are classified as opportunistic infections are caused by excessive growth of the fungal genus Candida. In the human body, Candida fungi can live as parasites or saprophytes in the mouth, respiratory tract, digestive tract, or vagina (Siregar, 2004). In reproductive physiology case studies, many women complain of flour albus (female vaginal discharge) and feel very uncomfortable, itching, smelly, sometimes even burning and redness. A lot of research is developing regarding the female reproductive organs, it turns out that it is related to daily habits. (Maharani, 2009).

One of the causes is the problem of cleanliness around the female sex organs. Leucorrhoea is a physiological thing if it occurs in the period before and after menstruation. Abnormal vaginal discharge can be caused by bacterial, fungal, and parasitic infections. Yeast infections of the vagina are most often caused by Candida, spp, especially Candida albicans (Brown and Chin, 2002). According to WHO estimates 1 in 20 adolescents in the world experience vaginal discharge every year.

The number of women in the world in 2013 was 6.7 billion people and who had experienced vaginal discharge around 75% at least once in their lifetime and 45% of them could experience it twice or more. In Indonesia, the number of women who experience vaginal discharge is very large, around 90% of women have the potential to experience vaginal discharge because Indonesia is a country with a tropical climate. Topical drugs that have been used to treat candidiasis include Nystatin, Clotrimazole, Miconazole, Ketoconazole, and other Azole groups. However, these antifungal drugs have limitations, such as severe side effects, a narrow antifungal spectrum, poor penetration of certain tissues, and the emergence of resistant fungi (Setyowati, 2013).

Therefore, it is necessary to find other safer alternative treatments. Sunkist orange is a fruit that is widely consumed by the public, sweet in taste, attractive appearance, easy to find anywhere, always available throughout the year (Naharsari, 2007). Citrus fruits as a source of nutrition contain vitamin C, fiber, sodium, folate, calcium, thiamine, niacin, vitamin B6, phosphorus, magnesium, copper, riboflavin, and pantothenic acid. Citrus fruits also contain secondary metabolites such as flavonoids, alkaloids, coumarins, limonoids, keratonids, and essential oils which have pharmacological activities such as anti-bacterial, anti-fungal, antioxidant, anti-inflammatory, anticancer, heart-protecting, neuroprotective and hepatoprotective (Lv et al., 2015).).

II. METHODS OF RESEARCH

Several systems are used as methods of which the tools used in this study are Petri dishes, ose, aluminum foil, blender, incubator, analytical balance (Vibra AJ), water bath, autoclave, rotary evaporator, disc paper, dropper pipette, centrifuge, test tubes, tube racks, calipers, and sterile cotton swabs.

The materials used were Sunkist orange peel, Sabaroud Dextrose Agar (SDA) medium, Candida albicans culture, 0.9% NaCl, 96% ethanol, and ketoconazole. Research procedure Sunkist orange peel extraction Sunkist oranges are washed with running water, then peeled the skin and cut into small pieces then spread on aluminum foil until the water is

absorbed until it dries for 3 days. After the Sunkist orange peel dries, the Sunkist orange peel is crushed until smooth, then weighed with an analytical balance.

Extraction of the simplicia powder produced was carried out by maceration using 96% ethanol solvent in a closed vessel, left in a cool place, protected from light, maceration was carried out in 3 stages. For the maceration results that have been obtained, a rotary evaporator is carried out at a temperature of ± 40oC, so that the ethanol is evaporated for 8 hours, then it is concentrated again with a water bath to obtain a thick Sunkist orange peel extract. Procedure: The inhibition test was carried out by the agar diffusion method using disc paper. The Candida Albicans stem was then taken 1-2 ose after that, scratched it on the surface of SDA media, and incubated at 37oC for 24-48 hours. After growing the colony, the colony was taken and suspended with a 0.9% NaCl solution of 5 ml into a test tube to measure its turbidity according to McFarland standards (Khafidhoh et al., 2015).

Dip a sterile cotton stick into the fungus suspension and press it on the tube wall until the cotton is not too wet, then rub it on the surface of the SDA media. The disc paper was soaked for 2 hours in each extract of Sunkist orange peel with a concentration of 20%, 40%, 60%, 80%, and 100%, aqua dest as a negative control and ketoconazole as a positive control. Then incubation at 37oC for 24 - 48 hours and after that measured the diameter of the clear zone formed around the disc paper in each group and measured with a caliper. Doing repeated application for four times (Simanihuruk, 2013).

This study is an in vitro study. This study used a laboratory experimental design (true experiment) with a post-test control group design only approach. The data of this study were tested for their meaning using the Kruskal Wallis non-parametric test followed by the Mann Whitney test. Data were processed with SPSS 16.0 for Windows.

III. Analyze and Results

Sunkist orange peel extract was made using the maceration method, which is a cold extraction process using 96% ethanol as a solvent. This method was chosen because it is the easiest method to do, does not require a lot of solvents, and the process of forming a thick extract is faster. In the extraction of 239.56 grams of dry powder of Sunkist orange peel, 50 ml of viscous extract was obtained.

3.1. Simplicia Screening and Characterization

The plants used were identified at the Herbarium Medanense (MEDA), the University of North Sumatra, namely the Sunkist citrus plant (Citrus Sinensis L. Osbeck) Kingdom Plantae (plant kingdom), division Magnoliophyta (flowering plants), class Magnoliopsida (seed plants), subclass Rosidae, order Sapindales, family Rutaceae, genus citrus, species Citrus Sinensis L. Osbeck. The results of phytochemical screening showed the presence of flavonoids, tannins, saponins, terpenoids, glycosides, and alkaloids. This is by the study of Lv et al. In 2015 which stated that oranges contain secondary metabolites such as flavonoids, alkaloids, coumarins, limonoids, keratonids, and essential oils which have pharmacological activities such as anti-bacterial, anti-fungal, antioxidant, anti-inflammatory, anticancer, protect the heart., neuroprotective, and hepatoprotective (Lv et al., 2015). The results of the phytochemical screening for the simplicity of Sunkist oranges can be seen in table 1.

TABLE 1. Phytochemical Screening Results for the Simplicia of Sunkist Oranges

No.	Secondary Metabolites	Results
1.	Flavonoid	+
2.	Tanin	+
3.	Saponin	+
4.	Steroid	-
5.	Terpenoid	+
6.	Glikosida	+
7.	Alkaloid	+

3.2. Inhibition Zone Test

In the effectiveness test of Sunkist orange peel extract against Candida Albicans, the extract concentrations are given were 5 series of concentrations, namely 20%, 40%, 60%, 80%, and 100% with aqua dest as a negative control and ketoconazole as a positive control. Inhibition zone diameter can be seen in table 2. Based on the inhibition zone data in table 2 it can be seen that the optimum concentration of Sunkist orange peel extract is at a concentration of 80% with a diameter of the inhibition zone is 14 mm. The mean diameter of the positive control zone of inhibition was 34 mm and the negative control was 0 mm. However, there was a decrease in the inhibition zone diameter at a concentration of 100%. This is thought to be a saturation factor. When the membrane/disc is saturated with the extract solution at a certain concentration, the membrane can no longer absorb the active substance from the extract.

TABLE 2. The Diameter of Inhibition Zone of Sunkist Orange Peel Extract on Growth of C. Albicans

K	N	U1	U2	U3	U4	Average inhibition zone diameter (mm)
P1	4	9,5	9	10	11	9
P2	4	12,5	12,5	12,5	11,5	12
P3	4	10,5	12,5	15	13	13
P4	4	13,5	13,5	15,5	13	14
P5	4	12,5	13	11,5	13,5	13
P6	4	35	34	34	34	34
P7	4	0	0	0	0	0

Information: K = Treatment Group, P1 = Cons. 20%, P2 = Cons. 40%, P3 = Kons. 60%, P4 = Cons. 80%,
P5 = Cons. 100%, P6 = Positive Control, P7 = Negative Control, U = Repetition

Based on Greenwood (1995), the inhibitory response of microbial growth by extracts can be seen from the diameter of the inhibition zone. If the diameter of the zone of inhibition is ≤ 10 mm, it is said that there is no inhibition, 11-15 mm of weak inhibition, 16-20 mm of moderate inhibition, and> 20 mm of strong inhibition. From this classification, the inhibition of the ethanol extract of Sunkist orange peel with a concentration of 40%, 60%, 80%, and 100% with an inhibition zone diameter of 12 mm, 13 mm, 14 mm, and 13 mm is classified in the category of weak inhibitory power and at a concentration of 10 % has no inhibitory power compared to positive control which has strong inhibition with an inhibition zone diameter of 34 mm.

Based on the Kolmogorov-Smirnov normality test, the measurement data for the diameter of the inhibition zone has a value of p-value (asymp. Sig.) = 0,000 which means that the distribution is not normal (p <0.05). Meanwhile, the Levene test results for the inhibition zone diameter have a p-value = 0.125. This means that the variance of the inhibition zone diameter data is homogeneous (p> 0.05). Because the distribution of data was not normally distributed and homogeneous, it was continued with the Kruskal Wallis Non Parametric test. The Kruskal Wallis test results showed that the value of P = 0.001, which means that there were significant differences in each treatment group (p <0.05). This significant difference indicates that the treatment that has been given has antifungal antifungal properties against the fungus Candida albicans. The comparison of the zone of inhibition in each group can be seen in the Post Hoc (Mann Whitney) test, which can be seen in table 3.

TABLE 3. Results of Mann-Whitney Test Data Analysis

K	P2	P3	P4	P5	P6	P7
P1	0,018*	0,043*	0,020*	0,021*	0,019*	0,014*
P2		0,442	0,017*	0,353	0,017*	0,011*
P3			0,189	1,000	0,019*	0,014*
P4				0,102	0,019*	0,013*
P5					0,019*	0,014*
P6						0,013*

Information: K = Treatment Group
Sign * indicates a significant value (p value ≤ α 0.05)

Based on the Mann-Whitney test, the results obtained were that there were significant differences in all groups except for groups P2 with P3, P3 with P4, P2 with P5, P3 P5, and P4 with P5.

The formation of the inhibition zone at the place where Candida albicans grow is caused by the content of alkaloids, flavonoids, saponins, and tannins from the Sunkist orange peel extract obtained from the phytochemical results carried out in this study. According to Abad et al. (2007), alkaloids can function as anti-fungi, one of which is alkaloid 2- (3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl) -1-methylethypentanoate which has activity against fungi. Candida. Alkaloids inhibit esterase, DNA, and RNA polymerase and can inhibit cellular respiration and play a role in DNA intercalation (Aniszewki, 2007).

Flavonoids have also been shown to be anti-fungal and have various biological activities (Chang, 2001). As an antifungal, saponins can damage the membrane in fungal cells, causing cell leakage which ultimately spurs cell death (Zhang et al., 2006). According to Jurenka (2008) research, tannins can inhibit the growth of Candida Albicans by disrupting the structure of the cell membrane and inhibiting the formation of cell walls in fungi (Hong et al., 2011).

Based on the results of the above research, the research hypothesis is accepted that the Sunkist orange peel extract can inhibit the growth of Candida Albicans fungi and the higher the concentration of the extract given, the larger the diameter of the inhibition zone formed.

IV. CONCLUSION

Based on the research results, it can be concluded that there is an antifungal effect of the Sunkist orange peel extract against the fungus Candida albicans. The optimal concentration of Sunkist orange peel extract in inhibiting the growth of Candida albicans fungi is at a concentration of 80% because the diameter of the inhibition zone formed is the largest, but according to the Greenwood classification, Sunkist orange peel extract has a weak inhibitory power.

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