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Original Research

THE EFFECTIVENESS OF ROSE FLOWER (ROSA CHINENSIS JACQ) ON CANDIDA ALBICANS COLONIES IN JELLY (SABOURAUD DEXTROSE AGAR) MEDIA

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ABSTRACT
Background: Approximately 90% of women in Indonesia have the potential to experience fluor albus. Fluor albus is mostly caused by Candida Albicans. Candida Albicans is a fungus that often causes infections on people. Herbal medicine is one alternative that can be used as a raw material for anti-fungial medication of Candida Albicans.
Objective: The purpose of this study was to determine effectiveness of rose flower (Rosa Chinensis Jacq) on Candida Albicans colonies.
Methods: This research was an experimental study with posttest only control group design, using four repetitions with a concentration of 7.5%, 10%, 12.5%, 15% and 17.5%. The hypothesis test used was One-way ANOVA (Analysis of Variance) with a significance level of 0.05.
Results: The result shows that 7.5% concentration, the growth of the colonies was 148.75 CFU/ml; at 10% concentration, the growth of the colonies was 123 CFU/ml; at 12.5%, the growth of the colonies was 86 CFU/ml, at 15%, the growth of the colony was 29 CFU/ml; at 17.5 %, the colony growth was 0, so it can be concluded the higher concentration of rose extract, the lower number of Candida Albicans colonies.
Conclusion: Rose extract (Rosa Chinensis Jacq) is effective in inhibiting the growth of the Candida Albicans fungus with minimum killing levels of 17.5%. Further studies on toxicity test on rose extracts on Candida Albicans are necessary.

Keywords: rose flower (Rosa Chinensis Jacq), Candida Albicans, Sabouraud Dextrose Agar

INTRODUCTION

Fluor albus is a very common symptom experienced by many women. About 90% of women in Indonesia have the potential to experience fluor albus. Research data on women's reproductive health shows that 75% of women in the world suffer from fluor albus at least once in their lifetime, 45% of them would experience this twice or more (Yatim, 2005).

Candida Albicans is one of the organisms function as normal flora in human bodies and is not dangerous. But Candida Albicans is also one of the funguses that cause infections on people. It is usually a local infection such as oral and vaginal infection (Chandranita, 2008; Simatupang, 2009). Candida Albicans is a facultative anaerobic organism that is capable of cell metabolism in both the anaerobic and aerobic atmosphere. This fungus grows at a temperature of 280C - 370C and at a pH of about 4.5-6.5. Candida Albicans will have fermentation process in an aerobic or anaerobic condition, which will ferment

glucose, maltose and sucrose, which will then produce acid and gas (Simatupang, 2009).

The result of previous research stated that gallic acid content on roses has antifungal effect against 17 kinds of fungi in concentrations of 3% (Tripathi et al., 2002). In addition, rose flower (Rosa chinensis Jacq) contains a lot of hydrolyzable tannins, flavonoids and anthocyanins (Cai et al., 2005). Flavonoid found in rose is about 41 mg / 100 g dry. Flavonoids, tannins and antioxidant activity found in roses can inhibit the growth of bacteria and fungi (Singh et al., 2009).

METHODS

Study design
This research was an experimental study with posttest only control group design using dilution tube test to determine the effects of roses antifungal (Rosa Chinensis Jacq) on Candida Albicans in Vitro.

Samples
The samples were vaginal candida albicans from Microbiology Laboratory of the University of Brawijaya cultured in a petri dish with SDA medium (Sabouraud dextrose Agar), using four repetitions for each extract concentration of roses (Rosa chinensis Jacq), which were 7.5%, 10%, 12.5%, 15%, and 17.5%.

Data collection
Stage 1
1) Making rose extract (Rosa Chinensis Jacq) using maceration method by providing 500g of fresh rose petals, dried in room temperature. Once dried, mashing them using the blender and weighing them, 2) Putting 100gr of dry sample into an Erlenmeyer glass with the size of 1 liter, soaking with methanol to a volume of 900 ml (3 times), then whisking until thoroughly mixed (± 30 min) and allowing to stand one night to settle, 3) Taking the top layer of the mixture of ethanol with active substances that had been drawn up and putting in the 1 lt evaporation flask, 4) Attaching the evaporation tube on evaporator and filling up the water bath with water, 5) Letting methanol solution separated from the existing active substance in the flask, and 6) Waiting until methanol flow stops dripping from the container flask (± 1.5 to 2 hours for 1 flask), and putting the result into a plastic bottle and storing it in the freezer.

Stage 2
1) Pre-test. It was conducted to find out the concentration of flower petals extract. On this test, the killing ability of each extract was the concentration of 5%, 7.5%, 10%, 12.5%, 15%, 17.5% with a ratio of (1 cc of aquabidest: 100) x concentration of the extract, 2) 65 grams of Saboraud Dextrose Agar powder was added to 70 ml of distilled water; stirred and covered it with aluminium foil and sterilized with autoclave together with the instruments used for 15 minutes with the temperature of 121ºC, 3) First layer of liquid Saboraud Dextrose Agar was poured into sterilized petri dish and let it to become solid, 4) Taking 1 ml of 1 ml Candida Albicans culture using heated osche above spiritus lamp until it was getting heated then letting it cool, 5) Giving 0.1 ml rose petal extract (Rosa Chinensis Jacq) with concentration of 7.5%, 10%, 12.5%, 15%, 17.5%, 6) Doing a full streaking on Saboraud Dextrose Agar media as much as 10µ1, 7) Petri dish was incubated for 24 hours in incubator with temperature of 37º, 8) Calculating the growth (Colony Counter) using Total Plate Count (TPC) method, and 9) Repeating the experiment as much as 4 times.

RESULTS

Test Result of the Growth of Candida Albicans

Minimum killing level was the lowest concentration of an antimicrobial that could kill fungi (characterized by the absence of bacteria growth in SDA medium) or colony growth of less than 0.1% of the number of colonies on initial inoculum (original inoculum / OI) in SDA medium by one ose streaking.

Table 1 Results of Calculation of Candida Albicans Colonies in Each Concentration

Repetition / Concentration	Growing Candida Albicans Colony				
	7.5%	10%	12.5%	15%	17.5%
I	149	127	96	31	0
II	149	115	70	26	0
III	146	115	62	25	0
IV	151	123	86	29	0
Mean	**148.75**	**123**	**86**	**29**	**0**

Based on the observation, it was found out that by the increasing concentration of rose petals extract, the number of colonies that grow on the SDA (Saboraud Dextrose Agar) was also increasingly reduced. 7.5% concentration resulted in the largest and most dense growth of fungal colonies, which was 149 CFU/l. Meanwhile, at a concentration of 17.5%, there was no growth of Candida Albicans colony.

From the result of isolated Candida Albicans colony growth and calculation, the killing ability minimum level of rose extracts could be determined, which was on the SDA with colony growth <0.1% of the original inoculum. The minimum killing level of rose extracts in this treatment was 17.5%. Prior to statistical analysis, to determine mean differences of each rose extract concentration, the normality and homogeneity tests were conducted (see Table 1).

The Results of Variant Data Analysis on the Number of Candida Albicans Colonies in Each Concentration

Figure 1 shows the significant reduction in the number of colonies in with rose extract provision, wherein the more number of extract concentration, the less number of Candida Albicans colonies were grown.

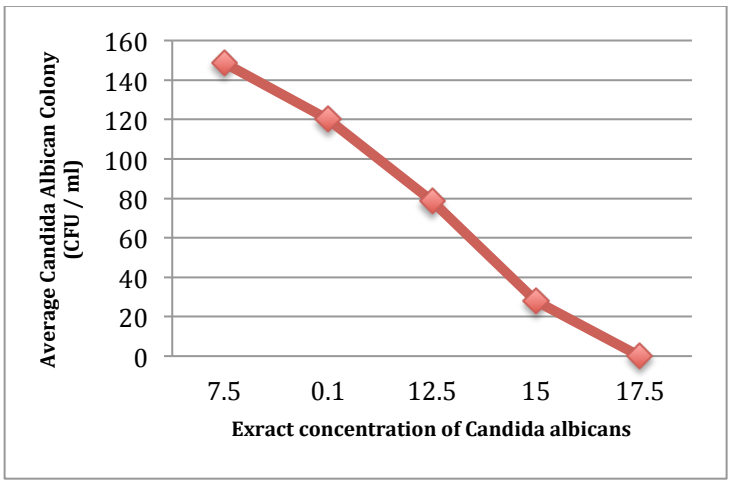

Figure 1 Average Number of Candida albicans Colonies in Each Concentration

Results of ANOVA test on Total Colonies of Candida Albicans in Each Concentration

From ANOVA test results in Table 2, it could be concluded that the more concentration of rose petals extract, the less number of growing Candida Albicans colonies. This shows that rose extracts (Rosa Chinensis Jacq) has anti-fungal potency against Candida Albicans.

Table 2 ANOVA Test Results for Candida Albicans Colonies

Concentration	N	(X)	SD	F	ρ
7.5%	4	148.75	2.062	270.439	.000
10%	4	120.00	6.000		
12.5%	4	78.50	15.351		
15%	4	27.75	2.754		
17.5%	4	.00	.000		

LSD (Least Significance Difference) Test Results on the Number of Colonies of Candida Albicans in Each Concentration

After ANOVA test, it was followed by LSD Post Hoc Test to determine which groups were different and which groups did not differ significantly in the number of colonies of Candida Albicans. Table 3 shows that there are differences in the concentration of each extract of rose petals with the average number of Candida Albicans colonies ($\rho = .000$, $\rho = .001$, $\rho < .05$).

Table 3 Different Test Results with LSD Post Hoc Test

Concentration		Average Difference (I-J)	ρ
Group (I)	Group (J)		
7.5%	10%	28.750*	.001
	12.5%	70.250*	.000
	15%	121.000*	.000
	17.5%	148.750*	.000
10%	7.5%	-28.750*	.001
	12.5%	41.500*	.000
	15%	92.250*	.000
	17.5%	120.000*	.000
12.5%	7.5%	-70.250*	.000
	10%	-41.500*	.000
	15%	50.750*	.000
	17.5%	78.500*	.000
15%	7.5%	-121.000*	.000
	10%	-92.250*	.000
	12.5%	-50.750*	.000
	17.5%	27.750*	.001
17.5%	7.5%	-148.750*	.000
	10%	-120.000*	.000
	12.5%	-78.500*	.000
	15%	-27.750*	.001

DISCUSSION

Rose extracts have antifungal effect against Candida Albicans that can be seen in table 1 showing that, the higher the concentration of rose extract used, the smaller the number of colony growth of Candida Albicans. It can also be stated that the higher the concentration of the rose extracts the higher antifungal effects. The result of One-Way ANOVA showed that there were differences between the mean value of colonies of Candida Albicans of each extract concentration rose (Rosa Chinensis Jacq). The LSD Post Hoc Test also showed a highly significant difference from all groups of rose extract concentration.

Rose extract has anti-fungal effect on the Candida Albicans fungus, which is caused by active substances that are soluble in methanol flavonoid. This is in line with previous

research, which states that there are hydrozable content of tannins, flavonoids and anthocyanins in roses (Cai et al., 2005). There are 17 types of flavonoids that have been identified and 7 types of flavonoid, which has not been identified at the roses, and there are 15 types of anthocyanins which are mostly classified as monoglicocides or diglicocides, cyanidin, pelargonidin and peonidin. Another study also states that roses contain ascorbid acid compounds, polyphenols, flavonoids and antioxidant activity (Roman et al., 2013). In addition, the content of vitamin C in rose is about 0.51 g / 100 g of dried roses, flavonoid about 41 mg / 100 g dried roses, and citric acid approximately 3.34 g / 100 g of dried roses. Vitamin C contains in roses decrease Candida albicans proliferation (Adamczak et al., 2012).

Flavonoids have antifungal effect, which is very effective in inhibiting cell growth (Orhan et al., 2010). Biological flavonoids activities were done by destroying the cell wall of Candida Albicans consisting lipid and amino acid which react with alcohol groups on a flavonoid compound that will break down the cell walls and the compound can enter the fungi cell nucleus. Furthermore, in the fungi cell nucleus, this compound will contact the DNA in Candida Albicans fungi cell nucleus and through differences in polarity between the lipids making up the DNA with alcohol groups on flavonoid compound, there will be a backlash that would damage the lipid structure of the DNA of the Candida Albicans. The activity of flavonoids is due to their ability to form complexes with the extracellular proteins, which is soluble with cell walls, so that microorganisms cannot attach and invade the host cell. Lipophilical flavonoids may also damage microbe membrane. Flavonoid compounds also inhibit topoisomerase II enzyme work on microorganisms associated with microorganism proteins (Van Melderen, 2001). DNA gyrase is one of topoisomerase II class of enzyme, DNA gyrase twists the strands of DNA and decipher DNA strands. The more lipophilic a flavonoid, the more ability to destroy the bacteria cell wall (Braner, 1993). Tannin contained in rose extract believed to have the same mechanisms as other phenolic compounds in inhibiting and killing the growth of fungi and bacteria and can react in an inactivation function way of genetic material. Tannin can also form complex compounds that are irreversible with proline, a complete protein that has the effect of inhibiting the synthesis of proteins to inhibit cell wall. Tannin also has the ability to inhibit reverse transcriptase enzyme from the microbial cells (Deacon, 1997).

In addition, Tannin can also inhibit the C-14 demethylase enzyme, which is also a catalase enzyme, which serves to spur ergosterol. Ergosterol forms a major component of the plasma membrane of fungi. With the disruption of this enzyme function then it cannot synthesize ergosterol normally. This causes plasma membrane structure does not form properly and the function is disrupted (Deacon, 1997).

CONCLUSION

Rose extract (Rosa Chinensis Jacq) is effective in inhibiting the growth of Candida Albicans fungus. The higher concentration of rose extract, the higher growth declining of Candida Albicans fungus. The minimum killing level of rose extracts (Rosa Chinensis Jacq) on Candida Albicans was 17.5%.

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REFERENCES

Adamczak, A., Buchwald, W., Zieliński, J., & Mielcarek, S. (2012). Flavonoid and organic acid content in rose hips (Rosa L., sect. Caninae DC. EM. Christ.). *Acta biologica cracoviensia series botánica, 54*(1), 105-112.

Braner, L. (1993). *Antimicrobial in Food*. New York: Marcel Dekker. Inc.

Cai, Y.-Z., Xing, J., Sun, M., Zhan, Z.-Q., & Corke, H. (2005). Phenolic antioxidants (hydrolyzable tannins, flavonols, and anthocyanins) identified by LC-ESI-MS and MALDI-QIT-TOF MS from Rosa chinensis flowers. *Journal of agricultural and food chemistry, 53*(26), 9940-9948.

Chandranita, A. (2008). *Gawat Darurat Obstetri Ginekologi dan Obstetri Ginekologi Sosial Untuk Profesi Bidan*. Jakarta: EGC.

Deacon, J. W. (1997). *Modern mycology*. London: Blackwell Scientific Publication.

Orhan, D. D., Özçelik, B., Özgen, S., & Ergun, F. (2010). Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiological research, 165*(6), 496-504.

Roman, I., Stănilă, A., & Stănilă, S. (2013). Bioactive compounds and antioxidant activity of Rosa canina L. biotypes from spontaneous flora of Transylvania. *Chemistry Central Journal, 7*(1), 73.

Simatupang, M. M. (2009). Candida albicans. Retrieved from http://repository.usu.ac.id/bitstream/handle/123456789/1935/09E01452.Pdf?sequence=1

Singh, S. D., Robbins, N., Zaas, A. K., Schell, W. A., Perfect, J. R., & Cowen, L. E. (2009). Hsp90 governs echinocandin resistance in the pathogenic yeast Candida albicans via calcineurin. *PLoS pathogens, 5*(7), e1000532.

Tripathi, G., Wiltshire, C., Macaskill, S., Tournu, H., Budge, S., & Brown, A. J. (2002). Gcn4 co-ordinates morphogenetic and metabolic responses to amino acid starvation in Candida albicans. *The EMBO Journal, 21*(20), 5448-5456.

Van Melderen, L. (2001). Molecular interactions of the CcdB poison with its bacterial target, the DNA gyrase. *International journal of medical microbiology, 291*(6-7), 537-544.

Yatim, F. L. (2005). *Penyakit kandungan: myoma, kanker rahim/leher rahim dan indung telur, kista, serta gangguan lainnya*. Jakarta: Pustaka Populer Obor.

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