EFFECTIVENESS OF COCOA (*Theobroma Cacao L.*) SEED EXTRACT ON THE GROWTH OF IN VITRO MALASSEZIA FURFUR

Fajar Daniswara Montana[1)], Yuni Setyaningsih[2)], Fajriati Zulfa[2)]

[1)]Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta
[2)] Department of Parasitology, Faculty of Medicine,
Universitas Pembangunan Nasional "Veteran" Jakarta

ABSTRACT

Background: *Pityriasis versicolor* or *Tinea versicolor* is a skin disease caused by the *Malassezia furfur* which is often found in Indonesia. People can use anti-fungal drugs to treat this disease. However, long-term use of anti-fungal drugs is relatively more expensive and can have side effects for its users. Cocoa bean husk contains flavonoids, saponins, and alkaloids which have anti-fungal effects. This study aimed to determine the antifungal effectiveness of the cocoa bean husk extract on the growth of *M. furfur*.
Subjects and Methods: This was an experimental study using cocoa bean husk extract with a concentration variance of 25%, 50%, 75%, 100%, with a positive control for ketoconazole 2% and a negative control using distilled water. The test was carried out by the well diffusion method using Sabouraud Dextrose Agar media. The inhibition of fungal growth was calculated by looking at the clear zone formed after 48 hours. Data were analyzed using Kruskal-Wallis and Post hoc Mann Whitney statistical tests.
Results: The mean diameter of the inhibition zone at a concentration of 25%, 50%, 75% and 100% was 3.42 mm, 4.07 mm, 4.9 mm, and 7.3 mm, respectively, and it was statistically significant (p = 0.001).
Conclusion: Cocoa bean husk extract has weak anti-fungal effectiveness at concentrations of 25%, 50%, and 75%, while at 100% it has moderate effectiveness.

Keywords: *antifungal, Pityriasis versicolor, cocoa bean shell, well diffusion, Malassezia furfur*

Correspondence:
Yuni Setyaningsih. Department of Parasitology, Faculty of Medicine,
Universitas Pembangunan Nasional "Veteran" Jakarta.

BACKGROUND

Indonesia is a country that has a tropical climate with high humidity, where this condition supports the growth of fungi that cause skin diseases (Pangalinan et al., 2012). Diseases caused by fungi are still often found in Indonesia because apart from having a tropical climate, Indonesia is also an area that is often hit by rain which creates a humid atmosphere for the development of fungi (Yusuf et al., 2017). According to data from the Indonesian Ministry of Health in 2012, there was an increase in the prevalence of skin diseases in all regions of Indonesia, which was 8.46% and continued to increase to 9% in 2013 (Depkes, 2013). *Pityriasis versicolor* is the most skin disease in Indonesia after dermatophytosis (Kristanty et al., 2009).

Pityriasis versicolor is a superficial fungal infection caused by lipophilic fungi from the genus *Malassezia* such as *M. globosa, M. sympodialis,* and *M. furfur* (Zarrab et al., 2015). The organisms that cause this disease are naturally present in human skin, but can cause clinical problems if the human where they live is exposed to risk factors such as warm temperatures, high humidity, immunosuppression, oily skin, and the use of corticosteroids. (Hawkins, 2014). The manifes-

https://doi.org/10.26911/the7thicph-FP.05.16

tation of pityriasis versicolor can be hypopigmentation, hyperpigmentation, can have erythematous lesions, above which form a fine scale, with normal skin surrounding the lesion site (Pramono and Soleha, 2018).

Malassezia furfur is a fungus that causes chronic infection of pityriasis versicolor in humans. *Malassezia furfur* is a lipophilic fungus that lives on human skin and hair follicles (Kauffman et al., 2011). Synthetic anti-fungal drugs used for the treatment of infectious diseases caused by fungi have recently developed further for their effectiveness, but often have serious effects if they are frequently used (Saifuddin, 2011). Anti-fungal drugs also have a relatively expensive price when used for a long period of time and contain chemicals that can cause side effects to users, so anti-fungal Study using natural ingredients needs to be developed (Karta and Burhanuddin, 2017).

Cocoa, which has the Latin name *Theobroma cacao L.,* is one of the leading commodities in Indonesia (Azizah et al., 2014). Cocoa cultivation in Indonesia reaches 1.6 million hectares and has a total production of around 500,000 tons of dry cocoa beans in 2011 (Mulyatni, et al, 2012). The processing of dry cocoa beans often results in a waste product in the form of cocoa bean shells. Due to the low economic power of cocoa bean shells, so far, its use has only been limited to providing food for livestock. Based on the results of phytochemical tests, cocoa bean shells contain active antimicrobial compounds such as alkaloids, flavonoids, and saponins (Kayaputri et al., 2014). Study conducted by Yumas (2017) proves that the active compound content of cocoa bean shell extract is capable of being an antibacterial agent for *Streptococcus mutans*. According to Freiesleben (2014) the active compounds of alkaloids, flavonoids, and saponins are structures that can kill or inhibit fungal development. On this basis, researcher interested in conducting study on the effects of skin extract cocoa *(Theobroma cacao L.)* against the growth of *Malassezia furfur* in vitro.

SUBJECTS AND METHODS

1. Study Design

This was an experimental study with a post-test control group design only. This study used extracts of cocoa bean shell *(Theobroma cacao L.)* with various concentrations which were tested for their effectiveness on the growth of *Malassezia furfur,* then compared between groups and with control groups as a comparison.

The cocoa bean shell extract is processed at the Bogor Medicinal and Spice Crops Study Institute (BALITTRO) with the maceration method using *N-Hexane* and 96% ethanol as a solvent. Phytochemical tests were also performed on the extraction results to determine the content of metabolite compounds contained in the extract. For testing the anti-fungal effect, the cocoa bean shell extract will be diluted with distilled water into 3 concentrations, namely 25%, 50%, and 75%, while the fourth concentration is 100% undiluted.

Positive control was ketoconazole 2% solution obtained by crushing 200 mg ketoconazole and dissolving it with 10 ml of sterile distilled water. The negative control in this study was sterile distilled water.

The method used in this test is the well method. First, the layer first of Sabouraud Dextrose Agar (SDA) media was made on a petri dish. After it hardens, a copper cylinder is placed to make a well hole in each SDA medium as a place to place the cocoa bean shell extract, positive control or negative control. In 1 petri dish 3 holes were made for a concentration of 25%, 50%, and 75%, while for a 100% concentration it was made with 1 separate petri dish. 2 holes were made for negative control in 1 petri dish, while for po-

https://doi.org/10.26911/the7thicph-FP.05.16

sitive control made with 1 separate petri dish. After that, the layer second of SDA media mixed with suspension was *M. furfur*. After that, wait 10-15 minutes for the media to harden, then remove the copper cylinder for the well, so that a pit is made on the SDA media. Then the extract, negative control or positive control is poured according to the Petri dish that has been made. This test is carried out 4 times. After that all the petri dishes were incubated at room temperature for 48 hours and the diameter of the clear zone formed using a digital caliper was calculated.

2. Data Analysis

The data obtained were then performed using Kruskal-Wallis and the Post hoc Mann Whitney test.

RESULTS

Inhibition of the fungal growth was assessed by measuring the clear zone formed outside the well using a caliper. In this study, the results of the average diameter of the inhibition zone can be seen in Table 1.

Table 1 shows that the negative control treatment group (aquadest) has an inhibition zone with an average of 0 mm, which means that it does not have an inhibition zone or does not have anti-effectiveness against *M. furfur*. In the positive control (ketoconazole) there was a picture of the inhibition zone with a mean of 38.87 mm. The inhibition zone formed in the cocoa bean shell extract was the smallest at a concentration of 25%, namely 3.42mm and the largest at a concentration of 100%, namely 7.3 mm.

The Kruskal-Wallis results can be obtained as shown in Table 2.

Table 1. Inhibition results of measurement of zone diameter

No.	Diameter of Constraint Zone around Well (mm)				Control (mm)	
	25%	50%	75%	100%	Control -	Control +
1	3.6	4.3	5.5	7.1	0	48.8
2	3.4	4.2	5.1	6.0	0	37.3
3	3.7	4.5	4.9	7.4	0	35.2
4	3.0	3.3	4.1	8.7	0	34.2
Average	**3.42**	**4.07**	**4.9**	**7.3**	**0**	**38.87**

Table 2. Diameter of inhibition zone of cocoa bean shell extract against *Malassezia furfur*

Test	p-value
Kruskal-Wallis	0.001

Based on these results, the p= 0.001 which means that there is a significant difference between treatment groups on the growth inhibition of *Malassezia furfur*. Post hoc (Mann-Whitney test) performed to determine which groups had significant differences. The results of the Post hoc Mann-Whitney test can be seen in Table 3.

Table 3. Analysis of *Post Hoc (Mann-Whitney)* Cocoa Bean Skin Extract on the growth of *Malassezia furfur*.

https://doi.org/10.26911/the7thicph-FP.05.16

	Groups	p-value
Control Negative	Control Positive	0.014
	25%	0.014
	50%	0.014
	75%	0.014
	100%	0.014
Positive Control	25%	0.021
	50%	0.021
	75%	0.021
	100%	0.021
25%%	50%	0.149
	75%	0.021
	100%	0.021
50%	75%	0.149
	100%	0.021
75%	100%	0.021

Based on the results of the test Post hoc Mann-Whitney, it can be seen that in the negative control group, positive control and 100% extract concentration with all test groups having a p value <0.05, which means they have different anti-fungal effectiveness. Meanwhile, between the concentrations of 25% with 50% and 50% with 75% there was no significant difference with p> 0.05, so it could be interpreted that these concentrations had the same antifungal effectiveness.

DISCUSSION

Based on the above study results, it appears that the extract of the cocoa bean shell has anti-fungal effectiveness. The extract of cocoa bean shell with the smallest concentration of 25% can inhibit the growth of *M. furfur*. The extract concentrations of 25%, 50%, and 75% resulted in an inhibition zone of 3.42 mm, 4.07 mm and 4.9 mm. The 100% extract concentration had an average inhibition zone diameter of 7.3 mm. These results indicate that the higher the concentration, the greater the resulting inhibition zone. This is possible because the secondary metabolites are contained more and more at higher extract concentrations.

The results of the Kruskal-Wallis statistical test indicated that there were significant differences between treatment groups, then the test was performed *Mann-Whitney Post hoc* to see the differences between groups. The extract group with a concentration of 25% with a concentration of 50% did not have a significant difference. This also occurred at the extract concentration of 50% and 75% which did not have a significant difference. These results are consistent with the description of clear zone inhibition at extract concentrations of 25%, 50% and 75% which results are <5mm and are included in the weak category according to the criteria of Davis and Stout (2009). The 100% extract concentration has a significant difference with all test groups, this is also in accordance with the clear zone inhibition description which results> 5 mm and is included in the moderate category according to the criteria of Davis and Stout (2009).

Cocoa bean shells have been studied for their effectiveness as an antibacterial against *Streptococcus mutans* by Yumas in 2017. In this study, the results showed that the cocoa bean shells were bacteriostatic against the growth of *Streptococcus mutans*. In terms of being an antifungal, Hutasoit (2019) conduc-

https://doi.org/10.26911/the7thicph-FP.05.16

ted Study on the effectiveness of cocoa bean shell extract on the growth of *Trichophyton rubrum*. The results of this study indicated that the cocoa bean shell extract had antifungal power against the growth of *Trichophyton rubrum*. These results are in line with this study, namely the extract of cocoa bean shell has effectiveness as an antifungal, in this case against *M. furfur*. The anti-fungal effectiveness produced by the cocoa bean shell extract comes from the secondary metabolites contained therein.

The anti-fungal effectiveness produced by the cocoa bean shell extract comes from the secondary metabolites contained therein. Based on the results of phytochemical tests conducted at the Indonesian Medicinal and Aromatic Plants Study Institute (BALITTRO), the extract of the cocoa bean shell contains flavonoids, alkaloids, and saponins. This is in line with the results of the phytochemical test conducted by Kayaputri (2014) which states that the cocoa bean shell extract contains these secondary metabolites. The mechanism of flavonoids as an antifungal is to denature fungal cell wall proteins so that the permeability of fungal cells increases and causes cell death (Andersen et al, 2016). Alkaloids as anti-fungal agents work by inserting cell walls and DNA, then preventing fungal DNA replication so that fungal growth will be disrupted (Dhamgaye et al, 2014). Saponins as anti-fungal agents act by building complexes with sterols, which are enzymes that make up fungal plasma cells, causing increased permeability of fungal cells (Arifin et al, 2018). Saponins are also said to inhibit or kill fungi by reducing the surface tension of the sterol membrane from the fungal cell walls, so that their permeability increases. This increased permeability can cause intracellular fluid that is more concentrated to be drawn out of the cell, resulting in nutrients, metabolic substances, enzymes, proteins in the cell, and the fungus to die (Hardiningtyas, 2009).

From this study it can be concluded that the extract of the cocoa bean shell *(Theobroma cacao L.*) has antifungal efficacy against growth *Malassezia furfur* in *vitro* by a weak antifungal power at a concentration of 25%, 50%, and 75% and antifungal power being at a concentration 100%.

REFERENCE

Andersen MO, Markham KR. (2016). *Flavonoids chemistry, biochemistry, and applications*. Taylor and Francis Group. New-York.; 416.

Arifin Z, Khotimah S, Rahmayanti S. (2018). Aktivitas Antijamur Ekstrak Etil Asetat Daun Mangga Bacang (*Mangifera foetida L.)* Terhadap *Candida albicans* Secara In Vitro. Jurnal Mahasiswa PSPD FK Universitas Tanjungpura.; 4(3): 1106-1119.

Azizah ND, Kumolowati E, Faramayuda F. (2014). Penetapan kadar flavonoid metode AlCl3 pada ekstrak metanol kulit buah kakao (*Theobroma cacao L.*). Kartika Jurnal Ilmiah Farmasi.; 2 (2): 45-49

Davis WW, Stout TR. (2009). Disc Plate Method of Microbiological Antibiotik Assay. Applied and Enviromental Microbiology.; 22(4): 666-670.

Depkes RI. (2013). Riset Kesehatan Dasar, Badan Penelitian dan pengembangan Kesehatan Kementrian Kesehatan RI, Jakarta.

Dhamgaye S, Devaux F, Vandeputte P, Khandelwal NK, Sanglard D., Mukhopadhyay G., Prasad R. (2014). Molecular Mechanisms of Action of Herbal Antifungal Alkaloid Berberine, in *Candida albicans*. *PLoS ONE.*; 9(8).

Freiesleben SH, Jäger AK. (2014). Correlation between plant secondary metabolites and their antifungal mechanisms-a

https://doi.org/10.26911/the7thicph-FP.05.16

review. Medicinal & Aromatic Plants.; 3(2) :154.

Hardiningtyas SD. (2009). Aktivitas Anti bakteri Ekstrak Karang Lunak *Sarcophyton sp*. yang difragmentasi dan tidak difragmentasi di perairan Pulau Pramuka, Kepulauan Seribu. SKRIPSI. FMIPA. IPB.

Hawkins DM, Smidt AC. (2014). Superficial Fungal Infections in Children. Pediatric Clinics of North America, 61(2): 443–455.

Hutasoit CMD. (2019). Uji Efektivitas Ekstrak Kulit Biji Kakao (*Theobroma cacao L.*) Terhadap Pertumbuhan *Trichophyton rubrum*. SKRIPSI. Universitas Pembangunan Nasional Veteran Jakarta.

Karta I, Burhanuddin. (2017). Uji Aktivitas Antijamur Ekstrak Akar Tanaman Bama (*Plumbago zeylanica*) Terhadap Pertumbuhan Jamur *Trichophyton mentagrophytes* Penyebab Kurap pada Kulit. Jurnal Media Sains.; 1(1): 23-31.

Kauffman CA, Pappas PG, Sobel JD. (2011). Dismukes WE. *Essentials of Clinical Mycology Second Edition*. Springer.; 245-248.

Kayaputri IL, Sumanti DM, Djali M, Indiarto R, Dewi LD. (2014). Kajian Fitokimia Ekstrak Kulit Biji Kakao *(Theobroma cacao L.). Chimica et Natura Acta*.; 2(1): 83-90.

Krisanty R, Bramono K, Wisnu I. (2009). Identification of Malassezia species from pityriasis versicolor in Indonesia and its relationship with clinical characteristics. Mycoses.; 52: 257-62.

Mulyatni AS, Budiani A, Taniwiryono D. (2012). Aktivitas antibakteri ekstrak kulit buah kakao (*Theobroma cacao L.*) terhadap *Eschericia coli, Bacillus subtilis, dan staphylococcus aureus*. Menara Perkebunan.; 80(2): 77-84.

Pangalinan RF, Kojong N, Yamlean PVY. (2012). Uji Efektivitas Ekstrak Etanol Kulit Batang Rambutan (*Nephelium lappaceum L.*) Terhadap Jamur *Candida Albicans* secara *In Vitro*. Jurnal Ilmiah Pharmacon.; Vol 1(1).

Pramono AS, Soleha TU. (2018). Pitiriasis Versikolor: Diagnosis dan Terapi. Jurnal Kesehatan dan Argomedicine.; Vol 5(1).

Saifuddin A. (2011). Standardisasi Bahan Obat Alam. Graha Ilmu; 1-11.

Yumas M. (2017). Pemanfaatan Limbah Kulit Ari Biji Kakao (*Theobroma cacao L*) Sebagai Sumber Antibakteri *Streptococcus mutans*. Jurnal Industri Hasil Perkebunan.; 12(2).

Yusuf AL, Nurawaliah E, Harun N. (2017). Uji efektivitas gel ekstrak etanol daun kelor (*Moringa oleifera L.*) sebagai antijamur *Malassezia furfur*. Kartika Jurnal Ilmiah Farmasi.; 5(2): 62-67.

Zarrab Z, Zanardelli M, Pietrzak A. (2015). *Tinea Versicolor (Pityriasis Versicolor),* European Handbook of Dermatological Treatments*:* 967–970.

https://doi.org/10.26911/the7thicph-FP.05.16