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INHIBITORY OF SOURSOP LEAVES (*Annona muricata* L.) EXTRACT AGAINST *Malassezia furfur* GROWTH

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ABSTRACT

Background: *Malassezia furfur in certain conditions can turn into a pathological phase, from the yeast phase to the mycelia phase that attacks the stratum corneum. Malassezia furfur is one of the causes of Pityriasis versicolor. The prevalence of pityriasis versicolor in Indonesia is quite high at around 40-50%. Several studies suggested that soursop leaves (Annona muricata* L.*) contains active substances with anti-fungal properties. This study aims to determine the inhibition of soursop leaves (Annona muricata* L.*) extract against the growth of Malassezia furfur.*
Methods: *This study was an experimental study with Post-test Only Control Group Design. Malassezia furfur fungi is used as subject in this study and ethanol extract of soursop leaves as a natural antifungal against Malassezia furfur.* The concentrations of extract tested were 100%, 80%, 60%, 30%. *Miconazole 2% was used as a positive control while DMSO 10% was used a negative one. The result of the study was analyzed by descriptive analysis which showed by increasing average diameter of antifungal.*
Results: *The results showed that soursop leaves (Annona muricata* L.*) extract had inhibitory effect on the growth of Malassezia furfur fungi at 100% concentration of 2.50 mm, 80% at 1.50 mm, 60% at 0.47 mm, 30% concentration at 0.25 mm and positive control of 14.50 mm. While as negative control, no inhibition zones were formed on SDA media.*
Conclusion: *Soursop leaves (Annona muricata* L.*) extract can inhibit the growth of Malassezia furfur, but the formed inhibition zone is weak. Further research is needed to found the best type of antifungal metabolites to maximize antifungal effects.*

Keywords: *Soursop leaves extract, Annona muricata L. , Malassezia furfur*

INTRODUCTION

Malassezia furfur is a lipophilic fungus that found from normal flora in the surface and of human skin. *Malassezia furfur* in certain conditions can turn into a pathological phase, from the yeast phase to the mycelia phase that attacks the stratum corneum. *Malassezia furfur* is one of the cause of *Pityriasis versicolor* [1].

Pityriasis versicolor is the most common infection. *Pityriasis versicolor* infection is most prevalent especially in tropical areas with hot and humid climates including Indonesia. The prevalence of *Pityriasis versicolor* in Indonesia is quite high at around 40-50%. *Pityriasis versicolor* is a harmless disease, but it can interfere with one's self-confidence due to hypopigmentation or hyperpigmentation of the skin and itching that made *Pityriasis versicolor* sufferers take medication [2].

Malassezia furfur was a commensal fungus that causes skin disease that was difficult to treat and requires long-term treatment because it was found in low socio-economic conditions and the level of personal hygiene was still low [1]. Treatment of *Pityriasis versicolor* can be either topical or systemic. Antifungal drugs that are often used are azole preparations, especially Miconazole, but they have side

effects such as irritation, burning and contact dermatitis, and can even cause drug resistance. Because of that, alternative medicine was needed in the form of herbal medicine. The herbal medicine that has been used was soursop leaves (*Annona muricata* L.) [3]. Soursop leaves contains saponins, tannins, and flavonoids. Flavonoids have phenolic compounds that are fungistatic or antifungal [4].

The previous study from Rohadi in 2016 stated that the antimicotic activity of soursop leaves ethanol extract against *Candida albicans* showed antimicotic activity with varied potential that depending on concentration [5]. The study from masloman et al in 2016 stated that the extract of 3rd soursop leaves from the branchlet tip that extracted by maceration method using 96% ethanol showed that the average inhibition zone diameter of soursop leaves extract (*Annona muricata* L.) on *Candida albicans* growth was 12.5 mm[6].

MATERIALS AND METHOD

This study was an experimental study with a post-test Only Control Group Design study by use *Malassezia furfur* as a study object, and use ethanol extract of soursop leaves (*Annona muricata* L.) which were used as a natural antifungal against *Malassezia furfur* [7]. The plants used in this study were soursop leaves (Annona muricata L.) originating from the city of Indramayu, West Java province. The experimental data were tested using descriptive analysis.

The population in this study was *Malassezia furfur* and the sample was pure isolates of *Malassezia furfur* fungi which were bred in the Microbiology Laboratory of FK Unswagati, Cirebon. Samples were divided into 100%, 80%, 60%, 30% of soursop leaves ethanol extract and negative control K (-) DMSO 10% and positive control K (+) Miconazole 2%. 100% concentration using 1 gram of soursop leaves extract and not diluted with 10% DMSO. only using the soursop leaf ethanol extract.

Malassezia furfur

Pure culture of *Malassezia furfur* was taken by inoculation loop, transferred into a test tube and added 1 ml of 0.9% NaCl until the turbidity equal to Mc Farland's standard (fungus concentration $1{,}5x10^8$ CFU / mL) [8]. Using the pour plate method, 1 ml of the dilution was taken, poured in Sabouraud Dextrose Agar (SDA) as growth media and then homogenized. It was incubated at 37 °C for 3 days [9].

SOURSOP LEAVES ETHANOL EXTRACT

Dried soursop leaves (500 g) were ground to powder and extracted (3-times) using ethanol (2 L) under reflux conditions. The extracts were combined and concentrated under vacuum at 40^0C with a rotavaporator to obtain the crude extract. The crude extract was dissolved in 10% dimethyl sulfoxide (DMSO) at a concentration of 100 mg/ml and stored at 30^0 C [6].

ANTIFUNGAL ACTIVITY BY DIFFUSION METHOD

Determination of inhibitory power in this research used the wells diffusion method with a diameter of 6 mm. Each well then dropped with soursop leaves extract (*Annona muricata* L.) which was diluted to 100%, 80%, 60% and 30% incubated at 37°C for 3 days. The antifungal potentials were determined by measuring the diameter of the inhibition zone (mm) [10].

RESULTS

The results of this study which were using 6 treatment with 100%, 80%, 60%, 30% concentration of soursop leaves extract (*Annona muricata* L.) and miconazole 2% as a positive control and DMSO 10% as a negative control with four replicates. The results of the diameter measurement of the inhibition (mm) in the table 1.

Table 1. Table of Average and Standard Deviation of Inhibition *Malassezia furfur*

The concentration of Soursop Leaves Extract (*Annona muricata* L.) (%)	Average ± SD (mm)	Inhibition of Antifungal Activity
100%	2.50 ± 0.57	Weak
80%	1.50 ± 0.57	Weak
60%	0.47 ± 0.05	Weak
30%	0.25 ± 0.05	Weak
K (-)	0.00 ± 0.00	No inhibition
K (+)	14.50 ± 0.57	Strong

The results showed with 100% concentration has an average antifungal diameter 2.50 ± 0.57 mm, 80% concentration with a diameter of 1.50 ± 0.57 mm, a concentration of 60% with a diameter of 0.47 ± 0.05 mm, a concentration of 30% with a diameter of 0.25 ± 0.05 mm and positive control with a diameter of 14.50 ± 0.57 mm, while in the negative control there was no inhibition or the inhibition zone is not formed on SDA media. The highest concentration on the growth of *Malassezia furfur* fungi was with 100% concentration with a diameter average of 2.50 ± 0.57 mm.

DISCUSSION

The average inhibition zone calculation of soursop leaves extract (*Annona muricata* L.) shows that the smallest inhibition zone diameter is 0.25 mm with a concentration of 30% and the largest inhibition zone at a concentration of 100% with a diameter of 2.50 mm. However, from all treatment groups, it was found that the largest inhibition zone was produced by positive control with a diameter of 14.50 mm. The inhibitory zones that are formed differently indicate that there is an ability of extracts that are different in inhibiting the growth of *Malassezia furfur* fungi which are influenced by the concentration of extracts from each group. The higher of the concentration of antifungal substances, the higher of the ability from these substances to inhibit the growth of *Malassezia furfur* fungi. This is in accordance with previous studies that, the effectiveness of antifungal agent (fungicide) is influenced by the concentration of the extract, the higher of the given of concentration will result in a wider inhibition zone, this is due to the many active substances contained in the extract [11]. The results of this study according to david and stout indicate that the administration of soursop leaves extract (*Annona muricata* L.) with a concentration of 100%, 80%, 60% and 30% has an antifungal effect but the formed inhibition is weak (weak the inhibition zone is about 0-4 mm. Soursop leaf extract has the potential as an antifungal but needs a step of purification first.

Soursop leaves (*Annona muricata* L.) contain secondary metabolites, one of which is phenol compounds can also damage the permeability of cell membranes so that it can lead to cell leakage [5]. Other compounds contained in soursop leaves (*Annona muricata* L.) are tannin, the mechanism of action of tannins by wrinkling and precipitating proteins from solutions by forming insoluble compounds, tannins play a role in the body's defense system and have anti-oxidant activity [12]. The content of the next compound is saponin, which is a surfactant which is polar in the form so that it will break the fat in the cell membrane which ultimately causes disruption of cell membrane permeability [13]. This results in the process of diffusion of materials or substances needed by the fungus can be disrupted, as a result of fungal cells can swell and rupture. The content of the next compound is flavonoids, the mechanism of action of flavonoids is to disrupt the process of food diffusion into cells so that the growth of fungi is stopped or the fungus dies [6]. Although it contains these compounds, soursop leaves extract has a weak inhibition. This might be occur because the secondary metabolites contained in the ethanol extract of soursop leaves (*Annona muricata* L.) are thought only be able to interact with fungal cell membranes but do not diffuse into the cell so that there is no interference with nucleic acid formation which will damage genetic material and result in disruption of fungal cell activity [14].

Kanazawa, inhibition of antifungal can be caused by the attachment of compounds to the cell surface or diffusion of these compounds into fungal cells [15]. Antifungal substances in an extract can inactivate the function of genetic material that is by interfering with the formation of nucleic acids (DNA and RNA). There are several factors that can affect antifungal activity, the concentration of antifungal, the number of fungi, the pH of the media, the incubation temperature, the potential for an antifungal in the solution tested, and the sensitivity of a fungus to the concentration of antifungi [14].

Tailor stated that soursop leaves (*Annona muricata* L.) produce various components of secondary metabolites, including annonaceous compounds, acetogenin, essential oils, reticulin, loreximine, coclaurine, annomurine and higenamine. Various types of secondary metabolites that are antifungal or antagonistic effects that will weaken the strength of secondary antifungal metabolites [16]. In contrast to Rohadi's statement, that at a concentration of 30% a inhibition zone of 14.64 mm was formed [5]. The inhibitory zone produced in our study seems unclear and there is no inhibition zone.

CONCLUSION

Based on the results of the study it can be concluded that the soursop leaves extract can inhibit the growth of *Malassezia furfur* fungi. the biggest inhibitory zone is formed at a concentration concetration of 100% which is equal to 2.50 mm, but is not effective as a traditional medicine. for further research it is recommended to examine the active substances contained in soursop leaves (*Annona muricata* L.) which can inhibit the growth of *Malassezia furfur* fungi.

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