

Antifungi Effect of Keghenyat Leaves Fraction (*Acmella uliginosa*) In Vitro Study

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Abstract

Introduction

Candida is a yeast fungus classes most commonly found in the oral cavity, gastrointestinal tract, reproductive tract and skin, especially the species *Candida albicans*. Alternative medicines such as herbs are easily available and affordable by the community, such as keghenyat leaves (*Acmella uliginosa*).

Aim of Study

To determine the activity of Keghenyat leaves fraction (*Acmella uliginosa*) compared to *Nystatin* against *Candida albicans* in vitro.

Methods

In vitro study was held in Laboratory of Genetics and Biotechnology Department of Biology, Faculty of Science and Medical Faculty, Sriwijaya University in December 2015 and January 2016. Research stages: extraction, fractionation, sensitivity test using *Nystatin* and *Candida albicans*, fractions antifungal activity test, the determination of minimum inhibitory concentration (MIC), bioautografi test and determination of compound classes. One final stages of testing the active fraction Keghenyat leaves (*Acmella uliginosa*) with *Nystatin*. Data were analyzed using ANOVA and Post Hoc Duncan test and linear regression using SPSS 20.

Results

Hexane fraction of Keghenyat leaves (*Acmella uliginosa*) is active against the fungus *Candida albicans*, has a Minimum Inhibitory Concentration (MIC) of 0.625% (6250 μ l) against the fungus *Candida albicans* antifungal contain active compounds that terpenoids and flavonoids, amounting to 84.68 mg / ml equivalent to 1 mg / ml *Nystatin* against the fungus *Candida albicans*.

Conclusion

There is a significance different MIC between hexane fraction of Keghenyat leaves (*Acmella uliginosa*) and *Nystatin*, *Nystatin* have more high activity.

Keyword : fraction, leaves *Keghenyat (Acmella uliginosa)*, *Candida albicans*, in vitro

Introduction

A fungal infection is called mycosis. One of the most common yeast infections is candidiasis. Candidiasis is a fungal infection caused by *Candida* sp. Although there are more than 150 species of *Candida*, but no more than 10 species are pathogenic in humans.¹⁻³ The Society tends to traditional treatment using herbs instead of using synthetic drugs. This is due to synthetic drugs are relatively expensive and can cause side effects such as kidney disorders, heart disorders and liver disorders.⁴ Utilization of natural medicine resources is possible in Indonesia, which is rich in various sources of flora. The use of natural sourced materials has relatively better healing rates when used correctly and appropriately, in terms of proper dosage, time of use, and how they are used.⁵

Oral thrush therapy using chemicals, such as Nystatin, will continuously cause the disease resistant to the drug. Side effects of oral nystatin include diarrhea, nausea, vomiting, and abdominal pain; while topical side effects of Nystatin include itching, burning, and skin and mucosal irritation.⁶ The side effects and the relatively expensive price of these drugs, hence sought alternative medicine in the form of herbs that are easily available and the price is affordable by the community, namely leaves keghenyat (*Acmella uliginosa*).⁷ Since ancient times, keghenyat leaves are often used as a medicine for fungal diseases, especially those caused by *Candida albicans*. It was hypothesize that the alkaloids contained in the keghenyat leaves disrupt the components of glucan, so that the cell wall layer is not completely formed and causes cell death and damages the fungal cell membrane.

Methods

This research was experimental laboratory conducted in vitro. The method used was the diffusion of agar by observing the inhibition zone formed around the dyed disk into various concentrations of the juice of the leaves of keghenyat against *Candida albicans* in the sample. This research will be conducted in Genetics and Biotechnology Laboratory of Biology Department Faculty of Science Sriwijaya University. This research was held in December of 2015 and January of 2016. The treatment group was taken on a tiered solubility level starting from the highest solubility of 40% then half taken and so on until the lowest solubility was 1.25%. The treatment groups were 20%, 10%, 5%, 2.5% and 1.25%. Treatment of the sample was four repetitions. The comparative group was Nystatin which was dissolved in a solution of dimethylsulfoxide (DMSO).

The extraction of keghenyat leaves used macerated methods. The extraction was soaked by using 1 liter of methanol solvent for 2x24 hours in the extractor, then filtered and evaporated using rotary evaporator until thickened paste to obtain extract methanol. The extract was fractionated by Liquid-Liquid Fractionation Method (FCC) with n-hexane, ethyl acetate and water methanol solvents gradually. Fraction (350 mg) dissolved in 0.5 mL ethanol as a solvent. The sample solution was bottled using capillary tube on Silica GF silent phase 254 which was activated by heating at 105°C - 110°C for

1 hour then eluted with methanol: chloroform phase (1:39) v/v. Chromatogram results were observed in UV 254 nm. Spotting was detected by H₂SO₄ spray.

Bioautography is used to detect the active compounds that have activity as antifungal. TLC eluted with mobile phase hexan: ethylacetate (8:2) v / v is aerated and placed for 20 minutes on the surface of SDA media in petri dishes that have been inoculated with 200 µL of fungus that have been made equivalent to 1.5 × 10⁸ CFU / mL. Data were analyzed using ANOVA and Post Hoc Duncan test and linear regression using SPSS 20.

Results

Table 1. Rendamen of keghenyat (*Acmella uliginosa*) Fraction

No	Solvents	Fraction (gram)	Persen berat (%)
1	n-hexan	22,4	43,7
2	etyl acetate	8,8	17,2
3	Methanol-water	20,0	39,1
Total		51,2	100

Table 1 showed that n-hexane fraction had a greater than ethyl acetate and methanol-water. The solvent has the ability to separate the compounds in the extract based on its polarity.

Table 2. Antifungal Activity of Keghenyat Fraction at Concentration 20% Against *Candida albicans*

No	Solvents	Repetition (mm)			
		1	2	3	4
1	Extract	0	0	0	0
2	n-hexan	12	11	12	12
3	etyl acetate	0	0	0	0
4	methanol-water	0	0	0	0

Table 2 showed that n-hexane fraction having mean inhibitory diameter on *Candida albicans* was 11.75 mm while ethyl acetate and methanol-water fraction did not have the inhibitory diameter for the fungus. N-hexane fraction had a strong inhibition to *Candida albicans*.

Table 3. Inhibitory Diameter n-Hexan Fraction of Keghenyat Leaves (*Acmella uliginosa*) Against *Candida Albicans*

Concentration of n-hexan fraction and nystatin (%)	Mean of Inhibitory Diameter (mm) ± SD
Nystatin 1	25 ± 0,57
20	13,5 ± 0,57*
10	12,5 ± 0,57*
5	11,5 ± 0,57*

2,5	10,5 ± 0,57*
1,25	9,25 ± 0,95*
0,625	6,25 ± 0,50*

* $p < 0,05$ against Nystatin 1% , Post Hoc Duncan ($p = 0,05$)

Table 3 showed that n-hexan fraction from concentration 20% - 0,625% had a significant difference in mean of inhibitory diameter than nystatin 1% . Nystatin 1% was more potent than n-hexan fraction from concentration 20% - 0,625% to inhibit fungus growth.

Table 4. Inhibitory Fungus Spora Growth n-hexan fraction of Keghenyat Leaves (*Acemella uliginosa*) Against *Candida Albicans*

Concentration of n-hexan fraction and nystatin (%)	Amount of Spora
Nystatin 1	$1,9 \times 10^6$
20	$8,9 \times 10^6$ *
10	$9,4 \times 10^6$ *
5	$9,7 \times 10^6$ *
2,5	$1,0 \times 10^7$ *
1,25	$1,0 \times 10^7$ *
0,625	$1,0 \times 10^7$ *

* $p < 0,05$ against Nystatin 1% , Duncan New Multiple Range Test (DNMRT) ($p = 0,05$)

Table 4 showed that n-hexan fraction from concentration 20% - 0,625% had a significant difference in amount of spora than nystatin 1% . Nystatin 1% was more potent than n-hexan fraction from concentration 20% - 0,625% to inhibit fungus spora growth.

Table 5. Phytochemical Test Keghenyat of Fraction (*Acemella uliginosa*)

No	Fraction	Eluen	Rf	Colouring	Active Compound
1	Hexan	Hexan:ethylacetate	0,58	Purple	Terpenoid
		(8:2)	0,20	Dark Yellow	Flavonoid

Phytochemical test showed hexan fraction contained active compound, terpenoid and flavonoid. Bioautography test showed the active compound, terpenoid and flavonoid, had potentiation to inhibit fungal growth.

Discussion

The chemical compounds contained in the Asteraceae family are terpenoids, flavonoids, and tannins. Family Asteraceae also contains essential oils, flavonoids, polyphenols and β -carotenoids.⁸⁻¹⁰ In this study it turns out that antifungal compounds obtained from keghenyat leaves (*Acemella uliginosa*)

were terpenoid and flavonoid compounds. Terpenoid work mechanisms and flavonoids can form complexes with ergosterol contained in the cell membrane of the fungus, the complex causes enlarged pores in fungal cells.¹¹ Through these pores is a small component of the contents of fungal cells out like nucleic acids and other proteins. It continues will cause the death of the fungus. The flavonoid complex is in a weak state, an indirect dissociation that causes the flavonoids to penetrate the cell.¹² At high concentrations in lipids, the greatest effect of flavonoids is its ability to join the cell lipid component. The mushroom cell membrane is composed of phospholipids which will cause the permeability of the cell membrane to be impaired so that the fungus is inhibited.¹³⁻¹⁵

Conclusion

There is a significance different MIC between hexane fraction of *Keghenyat* leaves (*Acmella uliginosa*) and *Nystatin*, *Nystatin* have more high activity.

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