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Research Article

CABBAGE LEAF EXTRACT (*BRASSICA OLERACEA VAR.* CAPITATA ALBA) AS A HERBAL MEDICINE FOR LEUKORRHEA

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ABSTRACT

Objective: This study was aimed to examine antifungal activity of cabbage leaf extracts against *Candida albicans*, to determine the minimum inhibitory concentration (MIC), to analyze the comparative value of the extracts with ketoconazole, and to study the fastest contact time to eliminate *C. albicans.*

Methods: The extraction of fresh cabbage leaf was done using the maceration method. The antifungal activity test and its comparative analysis against ketoconazole were assessed using the agar diffusion method. The extracts were tested for determining MIC value using solid medium, while the fastest contact time test was performed using turbidimetric method.

Results: Based on its inhibitory diameter, cabbage leaf extracts gave potent antifungal activity against *C. albicans*. The MIC of the cabbage leaf extract was between range 1.50% to 1.75%. A comparative analysis of the extracts with ketoconazole showed that ketoconazole gave greater antifungal activity than the extract at the same concentration. Cabbage leaf extract with concentration 2.5% gave the fastest contact time (2.5 minutes) for eliminating *C. albicans*, while cabbage leaf extract with concentration 0.4% gave the longest contact time (15 minutes) for eliminating *C. albicans.*

Conclusion: Cabbage leaf extract has a potential antifungal activity against *C. albicans* and prospective to be developed as a topical herbal medicine for treating candidiasis.

Keywords: *Brassica oleracea* var. capitata alba, Cabbage, *Candida albicans*.

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INTRODUCTION

Leukorrhea or flour albus or vaginal discharge was a symptom related to female genital disorders. It was white, yellowish-white, or gray color excessive fluid that discharges from vagina and sometimes smells offensive. Normally, women from all ages can have flour albus, but it could not be taken lightly, because this could lead to dangerous diseases. There are many causes of vaginal discharge (flour albus), it could be non-pathogen or pathogen condition. Pathologically, it might be caused by bacteria, fungus, virus, or parasite infection [1-3].

Flour albus that caused by bacteria could be treated by antibiotic; however, antibiotic consumption in a long term could be decreased vaginal normal flora, and in this condition, the fungus could grow in that area. *Candida* sp. was reported to be the most widely fungus that could be found at vaginal discharged, and from a hundred species of *Candida* sp that isolated from this discharge, 50-60% was *Candida albicans* [1].

Candidiasis is a fungal infection caused by yeasts that belong to the genus *Candida*. There are over 20 species of *Candida* yeasts that can cause infection in humans, the most common of which is *C. albicans*. *Candida* yeasts normally reside in the intestinal tract and can be found on mucous membranes and skin without causing infection; however, overgrowth of these organisms can cause symptoms to develop. Symptoms of candidiasis vary depending on the area of the body that is infected [3]. Patients with impaired immunity, such as those who have AIDS or are neutropenic as a result of cancer therapy, are at particular risk of developing *C. albicans* infections, which may become systemic [4-7].

Successful therapy for serious systemic *Candida* infections requires initiation of antifungal therapy as early as possible, as soon as adequate culture results are obtained [7,8]. Different classes of antifungals are now available to manage any type of *Candida* infection. Azoles, fluconazole in particular have become the mainstay of therapy over the past few years. Azoles inhibit the cytochrome P450 enzyme 14-α-sterol-demethylase. This enzyme is implicated in the biosynthetic pathway of ergosterol, which is an essential molecule of the fungal cell membrane. Inhibition of this enzyme leads to accumulation of 14-a-methylsterols on the fungal surface, which results in the arrest of fungal growth [9]. However, it had been reported that *C. albicans* was resistant against azole drugs so that it was necessary to seek the new drugs that were effective to treat candidiasis [2,10-12].

White cabbage (*Brassica oleracea* var. capitata alba) is a cruciferous vegetable used worldwide as a food and in traditional medicine. People had been using cabbage leaf to treat flour albus. Conventionally, people boiled cabbage leaf, and the boiling water was used to wash the vagina, and it could reduce itchiness that caused by *Candida* infection [13-15].

This study was aimed to determine antifungal activity, and minimum inhibitory concentration (MIC) from cabbage leaf extract against *C. albicans*, to analyze the comparative value of the extracts with ketoconazole, and to study the fastest contact time for cabbage leaf extract to eliminate *C. albicans*.

MATERIALS AND METHODS

Materials

The fresh cabbage leaf was obtained from cabbage leaf plantation in Lembang, West Java, Indonesia, and submitted to Plant Taxonomy Laboratory, Department of Biology, Universitas Padjadjaran for authentication. *C. albicans* ATCC 10231 and sabouraud dextrose agar (SDA) were obtained from Oxoid, Tokyo, Japan. Dimethyl sulfoxide (DMSO)and toluene were analytical grade and were produced by Merck, Germany; Ketoconazole was made by PT. Kimia Farma Jakarta, Indonesia.

Preparation of extracts

The fresh cabbage leaf that obtained from vegetables plantation in Lembang, West Java, and the plants was determined at Plant Taxonomy

Laboratory, Department of Biology, Universitas Padjadjaran. The fresh cabbage leaf was cut into small pieces and then macerated using ethanol 70% as a solvent, for 3×24 hrs, and the solvent was replaced every 24 hrs. The liquid cabbage leaf extract was collected, and the solvent was evaporated using rotary evaporator to obtain thick cabbage leaf extract.

Antifungal activity test of cabbage leaf extract against *C. albicans*

Antifungal activity from cabbage leaf extract against *C. albicans* was examined using agar diffusion method. SDA was used as a medium for *C. albicans*. SDA (65 g) was suspended with one liter purified water and then heated with frequent agitation and boiled for 1 minute to completely dissolve the medium. SDA medium was sterilized in the autoclave at 121°C for 15 minutes, and then, let it cool to 45-50°C. *C. albicans* was added into the SDA medium and then poured the mixture of SDA medium and *C. albicans* into petri dishes. Sterile filter paper discs (about 6 mm in diameter) were impregnated with 10 µl of cabbage leaf extracts with concentration 20%, 40%, 60% dan 80% in DMSO. The above 4 discs were applied into the mixture SDA medium *C. albicans* and then incubated at 37°C for up to 48 hrs. When growth took place, the size of zones of inhibition was measured for each antifungal agent [16].

Determination of MIC

Cabbage leaf extract was diluted with SDA medium to obtain cabbage leaf extract concentration 10; 5; 2.5; 2.25; 2; 1.75; 1.5; 1.25; 1; 0.5; and 0.25%, then 5 ml of each mixture were poured into petri dishes. Allow the agar to set and then dry the surface of the plates. The *C. albicans* inoculant (5 µl) was applied to the plates using spreader. Then, let the petri dishes at 37°C for up to 48 hrs incubation. The growth of *C. albicans* colonies was observed. MICs of cabbage leaf extract are defined as the lowest concentration of cabbage leaf extract that will inhibit the visible growth of *C. albicans* colonies [16-18].

Antifungal activity ratio against *C. albicans* of cabbage leaf extract compared to ketoconazole

Antifungal activity ratio from cabbage leaf extract against *C. albicans* compared to ketoconazole was also examined using agar diffusion method. SDA medium was sterilized in the autoclave at 121°C for 15 minutes, and then, let it cool to 45-50°C, added with *C. albicans* and poured into petri dishes. Sterile disks were impregnated with 10 µl of cabbage leaf extracts or ketoconazole with various concentrations in DMSO. The diameter of bacterial growth inhibition zone was measured by calipers and then plotted as an ordinate (Y) at the graphic and the axis (X) was the log of extracts concentration. Based on the graphic, the linear equations for both cabbage leaf extract and ketoconazole were determined. The comparison of antifungal activity from cabbage leaf extract and ketoconazole was calculated using this equation [16].

Determination of the contact time for cabbage leaf extract to eliminate *C. albicans*

The contact time of the cabbages extract to kill or eliminate *C. albicans* was determined using turbidimetric method. Cabbage leaf extract was diluted in DMSO to form concentration 1.75; 2; 2.25; 2.5; and 3% b/v. Let the *C. albicans* inoculant contact with each cabbage leaf extract concentration at the various time from 2.5 to 15 minutes. The inoculants then were added into liquid medium consisting broth and dextrose. Then, let the mixture at 37°C for up to 18-24 hrs incubation. The growth of *C. albicans* in the liquid medium was observed and compared with positive control (*C. albicans* inoculant added into liquid medium) and negative control (liquid medium only) [16].

RESULTS AND DISCUSSION

Preparation of extracts

Based on the result of the determination of the plant, the plant material was from *B. oleracea* var. Capitata alba, Family Brassicaceae. The rendemen of fresh cabbage leaf extract that obtained from maceration method with ethanol 70% as a solvent was 5.54%. The amount of the extract is small, because the material plants that we used in this experiment was fresh cabbage that still contains a large amount of water. The water content of the thick cabbage leaf extract was 16.6%.

Antifungal activity test of cabbage leaf extract against *C. albicans*

The cabbage leaf extract at the concentration 20%, 40%, 60%, and 80% has antifungal activity against *C. albicans*. The higher cabbage leaf extract concentrations yielded bigger diameter of inhibition against *C. albicans*. Table 1 shows the diameter of inhibition zone from various concentrations of cabbage leaf extract from day 1 to day 4.

Determination of MIC of cabbage leaf extract

MICs of cabbage extract are defined as the lowest concentration of cabbage leaf extract that will inhibit the visible growth of *C. albicans* colonies. The MIC test in this experiment was carried out using solid method. The concentration of cabbage leaf extract for this MIC test was in range 0.25-10%. The *C. albicans* inoculant was spreaded at the top of the mixture of SDA medium and various concentration of cabbage leaf extract. Table 2 shows the results of observation of *C. albicans* growth at the mixture of SDA medium and cabbage leaf extract at various concentrations.

There was still the growth of *C. albicans* colony on the SDA medium with 1.50% cabbage leaf extract, but there were no sign of *C. albicans* colony on the SDA medium with 1.75%. Based on this data, the MICs of cabbage leaf extract against *C. albicans* were between concentration range 1.50-1.75%.

Antifungal activity ratio against *C. albicans* of cabbage leaf extract compared to ketoconazole

The comparison test of cabbage leaf extract and ketoconazole antifungal activity against *C. albicans* was carried out to compare the cabbage leaf extract with antifungal drugs that had been widely used in the society. Ketoconazole is one of the antifungal drugs of choice. Table 3 shows the diameter of inhibition zone of ketoconazole against *C. albicans*. Fig. 1 shows the effect log of various doses of ketoconazole against diameter of inhibition zone of Candida albicans.

The linear equation of ketoconazole antifungal activity from the data above was y=1.3 x +19. The diameter of inhibition zone for ketoconazole against *C. albicans* at the dose 400 µg/50 µl or log dose 2.602, based on the linear equation, was (Y) 22.5 mm. The antifungal

Table 1: The diameter of inhibition zone of cabbage extract against *C. albicans*

Day	Diameter of inhibition zone (mm)			
	20%	40%	60%	80%
1	0.00	0.00	0.00	0.00
2	16.10	17.10	18.50	20.20
3	16.20	17.20	18.50	20.20
4	16.20	17.20	18.60	20.20

C. albicans: Candida albicans

Table 2: The observation results on *C. albicans* growth at various concentrations of cabbage leaf extract

Concentration of cabbage leaf extract (%)	The growth of *C. albicans* colony
0.25	+
0.50	+
1.00	+
1.25	+
1.50	+
1.75	-
2.00	-
2.25	-
2.50	-
5.00	-
10.00	-

"+" means there were colony of *Candida albicans* on the media, "-" means there were no colony of *Candida albicans* on the media, *C. albicans: Candida albicans*

Table 3: The diameter of inhibition zone of cabbage extract against *Candida albicans*

Concentration (%)	Doses (µg/50 µl)	Log of dose	Diameter of inhibition zone (mm)
0.8	400	2.602	24.00
0.6	300	2.477	23.00
0.4	200	2.300	22.00
0.2	100	2.000	20.00

C. albicans: Candida albicans

Table 4: The impact of various concentrations of cabbage leaf extract toward the contact time to eliminate *C. albicans*

Cabbage extract concentration (%)	Contact time (minutes)					
	2.5	5	7.5	10	12.5	15
3.00	-	-	-	-	-	-
2.75	-	-	-	-	-	-
2.50	-	-	-	-	-	-
2.25	+	+	-	-	-	-
2.00	+	+	+	-	-	-
1.75	+	+	+	+	+	+

- = clear, + = muddy because of the growth of *Candida albicans*,
C. albicans: Candida albicans

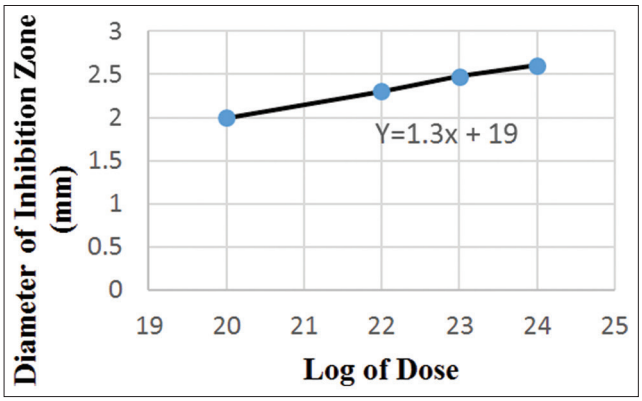

Fig. 1: Graphic of effect log dose of ketoconazole against diameter of inhibition zone of *Candida albicans*

activity of cabbage leaf extract was treated the same as the data of antifungal activity of ketoconazole. The linear equation of cabbage leaf extract antifungal activity was y=1.183x + 14.92. To produce the same diameter of inhibition zone (Y=22.5 mm), this Y value was put in cabbage leaf extract linear equation, and the log dose (X) was 6.1961. The antilog of 6.1961 was 1570766.32. Hence, the dose of cabbage leaf extract that will produce 22.5 mm diameter of inhibition zone was 1570766.32 µg/50 µl. Hence, the comparison of the doses from ketoconazole and cabbage leaf extract was 1: 3926,9158.

Determination of the contact time for cabbage leaf extract to eliminate *C. albicans*

The contact time was the time period for certain concentration of cabbage leaf extract to eliminate *C. albicans*. Table 4 shows the impact of various concentrations of cabbage leaf extract toward the contact time to eliminate *C. albicans*.

Based on the data in Table 4, cabbage extract with concentration 2.5% gave the fastest contact time (2.5 minutes) for eliminating *C. albicans*, while cabbage extract with concentration 0.4% gave the longest contact time (15 minutes) for eliminating *C. albicans*.

CONCLUSION

The result of the experiment showed that cabbage extracts with concentration 20%, 40%, 60%, and 80% gave antifungal activity; the MICs of cabbage extract against *C. albicans* were between 1.5% and 1.75%, cabbage extract's antifungal activity ratio against ketoconazole was 1: 3926,9158. Cabbage extract with concentration 2.5% gave the fastest contact time (2.5 minutes) for eliminating *C. albicans*, while cabbage extract with concentration 0.4% gave the longest contact time (15 minutes) for eliminating *C. albicans*. From the results above from this experiment, cabbage leaf extract has a potential antifungal activity against *C. albicans* and prospective to be developed as a topical herbal medicine for treating candidiasis.

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