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COMPARISON OF EFFECTIVENESS BETWEEN CELERY JUICE (*Apium graveolens* L.) AND 2% MICONAZOLE TOWARDS THE GROWTH OF *Malassezia furfur*

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ABSTRACT

***Introduction**: Pityriasis versicolor is caused by the fungi Malassezia furfur with a worldwide prevalence of 50%, including tropical countries, second only to dermatitis in Indonesia. Pityriasis versicolor is difficult to treat and requires long-term treatment. The disease has high recurrence risk and may cause drug resistance. 2% Miconazole is known to have long-term side effects; therefore, alternative treatment is needed. Several studies suggested that celery (Apium graveolens L.) contains active substances with anti-fungal properties. This paper aims to investigate the comparison of effectiveness between celery juice and 2% Miconazole towards the growth of Malassezia furfur.*
***Methods**: This is an in-vitro experimental study with post-test only control group design. The subjects were split into 5 groups which were given celery juice in 10% DMSO with the concentration of 10%, 20%, 30%, 40%, and 50%. A negative control group was given only 10% DMSO and the positive control group was given 2% Miconazole. The data were then analyzed using Kruskall-Wallis followed by Mann-Whitney test.*
***Results**: The Kruskall-Wallis test showed all concentration of celery juice had antifungal effect with $p=0.000$ ($p<0.05$) and were effective in inhibiting the growth of Malassezia furfur. The Mann-Whitney test showed that the 50% celery concentration was as effective as 2% Miconazole in inhibiting the growth of Malassezia furfur ($p=0.495$).*
***Conclusion**: Celery juice (Apium graveolens L.) was effective in inhibiting the growth of Malassezia furfur with 50% concentration as the most effective concentration.*

***Keywords**: Apium graveolens L., squeezed celery juice, Malassezia furfur, Miconazole 2%*

INTRODUCTION

Malassezia furfur is a normal flora on the skin. *Malassezia furfur* in certain condition can develop from the yeast phase to the pathological mycelia that invades the stratum corneum. *Malassezia furfur* is one the cause for Pityriasis Versicolor (PV) or Tinea Versicolor. Pityriasis is one of the most common infection worldwide [1,2], especially in humid tropical climate where the prevalence is 50% in tropical country while only 1.1% in colder climate such. [3–5]. PV has a high incidence rate in Indonesia and the prevalence is second after dermatitis [6].

Malassezia furfur is a skin disease-causing lipophilic fungus that is hard to treat and require long term medication and often found in patients with lower socioeconomic status and closely related to the level of personal hygiene [2,7].

Treatment for *Malassezia furfur* may be topical or systemic [7]. Topical treatment does not specifically act on Malassezia as topical treatment only remove dead infected tissues [8,9]. Systemic treatment has broader effect and able to treat recurrent infection, but failure in topical treatment may cause high chance of relapse and often requires intermittent prophylactic treatment [10]. Miconazole is one of the most widely used topical drug for antifungal [11]. Side effects of azol-based antifungal medication such as miconazole includes mild burning or itching, swelling, skin irritation, tenderness, flaking, and increased urination [10,12]. Based on previous research, Miconazole was observed to be resistance in all candida isolates by 30% tested respectively [13].

Herbal treatment is often used as an alternative therapy due to less side effects [14,15]. Some herbals have antifungal effect to *Malassezia furfur*, such as clove (*Syzygium aromaticum*), henna leave (*Lawsonia inermis* L.), fingger roots (*Boersenbergia pandurata* Roxb.) and Cerely (*Apium graveolens* L.) [15–17]. Celery is easy to obtain and relatively cheap. Celery can be squeezed to obtain its natural juice without any additional water required so it is easy to made, comparing with extract and essetial oil. Celery is known to have antifungal properties with components such as atsiri oil (limonene), flavonoid (apigenin, isoquercetrin), saponin, and tannin [18–20]. This study aims to compare the antifungal property of squeezed celery juice with 2% miconazole to the growth of *Malassezia furfur*.

MATERIALS AND METHODS

This is an in vitro laboratory experiment with post-test only control group design with *Malassezia furfur* as test samples. Sample were pure isolate of Malassezia furfur grown at Faculty of Medicine Universitas Swadaya Gunung Jati Microbiology Lab. Samples were divided into 5 groups, (P1), (P2), (P3), (P4), and (P5) which were given squeezed celery juice with increasing concentration of 10%, 20%, 30%, 40%, and 50% respectively.

There were insufficient data about the effective antifungal concentration of squeezed celery juice (*Apium graveolens* L.) toward *Malassezia furfur*. The study done by Ardelia et al., (2010) that observed the antifungal activity of squeezed celery juice (*Apium graveolens* L.) toward Candida albicans in 25%, 50% and 100% concentration, showed that 50% concentration of squeezed celery juice (*Apium graveolens* L.) has the most inhibitory activity[20]. The study done by Nitihapsari (2010) showed that 50% of celery extract has the same effectiveness in inhibiting *Malassezia furfur*, compared with Ketokonazole 2% [21] This study used the concentration less than 50% of squeezed celery juice (*Apium graveolens* L.) to know the minimum concentration that still have the inhibitory activity.

Two control groups were utilized K(-) using 10% Dimethyl sulphoxide (DMSO) and K(+) using 2% miconazole. The experiment was done in quadruplicate. The experimental data were tested for normality using Shapiro-Wilk test followed by analysis with Kruskall-Wallis and Mann-Whitney post-hoc analysis.

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 108 CFU / mL) [18]. Using pour plate method, 1 ml of the dilution was taken, pured in Sabouraud Dextrose Agar (SDA) as growth media and then homogenized. It was incubated at 37 °C for 3-4 days [19].

Celery juice (*Apium graveolens* L.)

The celery juice was made by separating the leaves and the stems of 1 kg fresh celery plants. They were washed under running water and let dry by aeration. The washed celery leaves were sliced into ± 2 mm, then crushed using a juicer. Then they were squeezed with a sterile gauze cloth to obtain the juice [18,20]. Around 100 ml of juice can be obtained from 700mg of dregs. Celery leaf juice then diluted with DMSO 10% based on the concentration.

Determination of Antifungal Potential

Determination of inhibitory power in this research used the wells diffusion method. The samples was incubated at 37°C for 3 days [19]. The antifungal potentials were determined by measuring the diameter of inhibition zone (mm) that was characterized by a clearness around the well [21].

RESULTS

From a measurement of antifungal activity at Table 1, it showed the squeezed celery juice with 10% concentration (P1) has an average antifungal diameter of 6.00 mm, (P2) with a diameter of 8.75 mm, (P3) with a diameter of 11.00 mm, (P4) with a diameter of 13.50 mm, and (P5) with a diameter of 15.25 mm. K(-) with 10% DMSO has an average antifungal diameter of 3.50 mm, while K(+) given 2% miconazole has an average of 15.50mm. The squeezed celery juice with the best antifungal property is the concentration of 50%, followed by 40%, 30%, 20%, and 10% respectively.

Table 1. Antifungal effectiveness of squeezed celery juice on *Malassezia furfur*

Celery concentration (%)	Diameter of antifungal activity (mm)				Average (mm)±SD	Antifungal Activity*	*p*-value
	PI	PII	PIII	PIV			
10% (P1)	5	7	5	7	6.00 ±1.15	Medium	0.000
20% (P2)	9	9	8	9	8.75±0.50	Medium	
30% (P3)	10	12	12	10	11.00±1.15	Strong	
40% (P4)	14	13	14	13	13.50±0.57	Strong	
50% (P5)	16	15	15	15	15.25±0.50	Strong	
K(-)	4	3	3	4	3.50±0.57	Weak	
K(+)	15	16	15	16	15.50±0.57	Strong	

*Kandoli et al., 2016 [22].

Normality test using Shapiro-Wilk showed p-value<0.05, which means the population is not normally distributed. Homogeneity test also showed p-value<0.05, which means the population is not homogeny. The data analysis was then continued using Kruskal Wallis. From Kruskal-Wallis test, it showed p-value of .000 which means that there was significant difference in antifungal activity of squeezed celery (*Apium graveolens* L.) between (P1), (P2), (P3), (P4), (P5), K(-), and K(+).

Table 2. Mann-Whitney post hoc test

	10% (P1)	20% (P2)	30% (P3)	40% (P4)	50% (P5)	K(-)	K(+)
10% (P1)							
20% (P2)	.017						
30% (P3)	.018	.017					
40% (P4)	.018	.017	.018				
50% (P5)	.017	.015	.017	.017			
K(-)	.018	.017	.018	.018	.017		
K(+)	.018	.017	.018	.018	.495*	.018	

*no significant difference (p>0.05)

Mann-Whitney test result showed no significant difference between 50% squeezed celery juice and 2% miconazole K(+) with p>0.05. This means that P5 has similar strength in antifungal activity with 2% miconazole.

DISCUSSION

Malassezia furfur is a spherical shaped yeast that has a distinguishing bottleneck at one end amd is the only species of fungi that are part of the human normal flora [18,22,23]. This study showed that the concentration of squeezed celery juice with the highest antifungal activity is 50% (P5). Similar

observation was also seen in Ardelia et al., (2010) with 50% squeezed celery juice forming a clear ring after inhibiting *C. albicans* growth in vitro due to their apigenin and other essential oils [20].

Mann-Whitney post hoc test showed no significant difference in antifungal activity observed between (P5) and 2% miconazole, suggesting that squeezed celery juice of 50% can be an alternative to 2% miconazole. Nitihapsari (2010) observed similar result when comparing squeezed celery juice of 50% with 2% ketoconazole in inhibiting the growth of *Malassezia sp*. Celery contains essential oils and flavonoids such as graveobiosid A (1-2%) and B (0.1-0.7%) that may act as antibacterial and antifungal agents [21]. A similar study by Rachmawati (2014) showed that ethanol extract of celery exhibits antifungal property toward *C. albicans* with 80% as the most effective concentration [24]. Celery *(Apium graveolens* L.) is believed to contain various antifungal substances such as flavonoid (1.7%), saponin (0.36%), tannin (1%) and essential oils (0.33%) [24].

Flavonoid is believed to have protein denaturing properties and acts to increase the membrane permeability of fungal cells and binds to proteins though hydrogen bonds causing disruption in the protein structure and eventually leads to cell lysis and cell death [25–27]. Another derivative of the flavonoid is apignenin, a water-soluble component showing antifungal activity towards *C. albicans* and *M. furfur* [24,25].

Celery essential oils such as limonene and saponin are also shown to have antifungal activities towards *C. albicans* and *M. furfur* [27–29]. Limonene is a terpene extracted with ethanol and believe to disrupt fungal cell membrane integrity and disrupting metabolic activity causing cell death [28,30–32]. Saponin is natural detergent and able to disrupt the integrity of the lipid bilayer of the fungal cell membrane [25,27,33].

The study can't consider that 50% concentration of squeezed celery (*Apium graveolens* L.) as the maximum antifungal activity. Hoewever, the 50% concentration has similar anti-fungal property as 2% miconazole. We suggest the next study to observe the higher concentration to know the maximum antifungal activity.

The ability of celery to inhibit fungal growth is similar to miconazole where miconazole inhibits the biosynthesis of ergosterol, causing cell lysis and directly attacking the fungal cell membrane [34].

CONCLUSION

Squeezed celery (*Apium graveolens* L.) exhibits potent antifungal activity that is similar to 2% miconazole, making it an suitable natural remedy for fungal infections.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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