

FORMULATION AND ANTIFUNGAL ACTIVITY OF PURPLE CABBAGE (*BRASSICA OLERACEA*) EXTRACT SPRAY AGAINST *CANDIDA* *ALBICANS* AS THE CAUSE OF ORAL CANDIDIASIS

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ABSTRACT

Purple cabbage (*Brassica oleracea*) is a plant known to have many health benefits, especially due to its abundant nutritional content, such as anthocyanins. One potential that is being developed is as a natural antifungal agent. This study aims to determine the effectiveness of a purple cabbage extract spray formulation in inhibiting the growth of *Candida albicans*, one of the fungi that causes oral candidiasis. The study was conducted experimentally using the disc diffusion method. Purple cabbage extract was obtained through a maceration process with 70% ethanol solvent. Antifungal activity tests were carried out using four different treatments, namely: 100% purple cabbage extract, 50% purple cabbage extract, 100% purple cabbage extract spray, and 50% purple cabbage extract spray. The test results showed an average inhibition zone of 1.43 mm with a weak category, 1.32 mm with a weak category, 5.33 mm with a moderate category, and 6.83 mm with a moderate category. This suggests that a spray formulation with a lower concentration is actually more effective, likely due to the more even distribution of the active compound across the surface of the medium. This research supports the potential use of natural ingredients, particularly purple cabbage, as an environmentally friendly and easily applied antifungal alternative in spray form.

KEYWORDS: Disc diffusion, Candidiasis, Yeast, Purple cabbage, Spray form.

INTRODUCTION

The mouth is the first organ in the digestive tract that plays a vital role in the processes of chewing, speaking, swallowing, and digesting food before it is passed on to the stomach (Amaniya *et al.*, 2023). Therefore, maintaining oral health and hygiene is crucial not only for the function of the mouth itself, but also for overall body health. Oral disorders, such as fungal and viral infections, and poor oral hygiene, can cause various health problems, including oral candidiasis (Lestari *et al.*, 2016). Oral candidiasis is one of the most common infectious diseases in the oral cavity and is caused by the overgrowth of *Candida albicans*.

Candida albicans is a fungus that naturally lives on the skin and mucous membranes of the body, including the oral cavity, without causing problems under normal conditions. However, when there is an imbalance in the microbiota or a weakened immune system, this fungus can overgrow and cause infections (Ok *et al.*, 2021). Some factors that trigger *C. albicans* overgrowth include excessive use of antibiotics, immune system disorders, and diseases such as diabetes. Symptoms include white patches on the tongue or mouth, pain, a burning sensation, itching, and difficulty swallowing. If left untreated, the infection can spread to other parts of the body and cause serious complications, especially in individuals with weakened immune systems.

The main treatment for oral candidiasis generally uses antifungal drugs such as nystatin, fluconazole, and ketoconazole which are effective in inhibiting the growth of *C. albicans* (Ok *et al.*, 2021). However, long-term use of these medications can cause side effects, such as digestive disorders, allergic reactions, and liver damage. Therefore, safe alternative treatments with minimal side effects are needed, one of which is the use of natural ingredients.

Purple cabbage is a plant known to contain various bioactive compounds, such as flavonoids, tannins, saponins, and steroids, which have antimicrobial and antifungal potential (Khairani *et al.*, 2024). Research shows that purple cabbage extract can inhibit the growth of *Candida albicans* with a significant inhibition zone using the paper disc diffusion method. A 30% extract concentration produces an inhibition zone with a diameter of 8.75 mm, which is considered moderate in its effectiveness (Khairani *et al.*, 2024).

One promising dosage form is a purple cabbage extract spray. Sprays facilitate direct application to the infected area in the oral cavity, ensuring even distribution and faster absorption than tablets or syrups (Nguyen & Hiorth, 2015). Furthermore, the spray's mucoadhesive properties allow the drug to remain at the site of infection longer, increasing therapeutic effectiveness (Atoosh & Ghareeb, 2024). The spray's advantages also make it particularly suitable for patients with swallowing difficulties, such as children and the elderly.

Based on this potential, this study aims to develop a formulation and test the antifungal activity of purple cabbage extract spray against *Candida albicans*, the cause of oral candidiasis. With this safe and effective natural formulation, it is hoped that it can become a practical, affordable, and side-effect-free alternative treatment for oral candidiasis.

MATERIALS AND METHODS

Sample Preparation

This research has been conducted in the Biology laboratory of Indraprasta PGRI University. The research was conducted experimentally using the disc diffusion method. Purple cabbage extract was obtained through the maceration method with 70% ethanol solvent. The tools used are glassware, autoclave, glass jars, 40 mesh sieve, gauze, aluminum

foil, blender, petri dish, refrigerator, vernier caliper, bunsen, ose wire, sterile cotton, scales, tweezers, micropipettes, laminar air flow, knife, oven, filter paper, test tubes, discs, spatula, mortar, pestle, funnel, stirring rod, and thermometer. Meanwhile, the materials used were distilled water, 70% ethanol solvent, *Candida albicans* ATCC 10231 isolate, 2% ketoconazole tablets, purple cabbage, SDA media (Sabouraud Dextrose Agar), SDB media (Sabouraud Dextrose Broth), bacteriological agar, methylated spirits, mint, glycerin and sorbitol.

The study used a Completely Randomized Design (CRD) with 6 treatments (Table 1), namely 100% purple cabbage extract (A), 50% purple cabbage extract (B), 100% purple cabbage extract spray (C), 50% purple cabbage extract spray (D), negative control (E), and 2% ketoconazole (F) as a positive control, each with 6 repetitions. Primary data were obtained from direct inhibition zone measurements in the Laboratory. Meanwhile, secondary data were obtained from previous publications *related to* the antifungal properties of purple cabbage extract, the mechanism of action of *C. albicans*, and oral candidiasis preparations with natural ingredients.

Table 1: Purple Cabbage Extract Spray Formulation (*Brassica oleracea*).

Material	Function	A	B	C	D	E
Sorbitol	Sweetener	0	0	2	2	0
Glycerin	Humectant emollient	0	0	3	3	0
Mint	Flavor	0	0	0.5	0.5	0
Purple Cabbage Extract	Active ingredient	20	10	20	10	0
Aquadest ad	Solvent	0	100	100	100	0
Amount		20	100	100	100	0

Making Simple Drugs

Fresh purple cabbage is selected, then cleaned to remove any dirt by washing it under running water, then dried and weighed. The cabbage is then sliced to transform it into a medicinal herb. To prevent the herb from spoiling, it is dried in a drying cabinet. After the drying process is complete, the herb is mixed and sieved using a sieve to produce a medicinal herb powder (Khairani *et al.*, 2024).

Making Purple Cabbage Extract

Purple cabbage extract was made through a maceration process of powdered simplicia with a ratio of 1:10. The sample was placed in a glass jar, then soaked in 75% ethanol solvent. The jar was tightly closed with aluminum foil and left for five days with stirring every six hours. After five days, the filtrate and residue were filtered using filter paper. The residue was then macerated again (remacerated) with the remaining 25% ethanol 70%, for two days with stirring every six hours. After two days, the material was filtered again to obtain the filtrate and residue. The first and second filtrates were then combined, poured, and filtered once more before being concentrated. The filtered filtrate was solidified using a vacuum rotary evaporator at 40°C until most of the solvent evaporated, resulting in a thick extract (Khairani *et al.*, 2024).

Media Creation

A total of 8.45 g of SDA powder was dissolved in 130 mL of distilled water (39 g/1000 mL of distilled water) in an Erlenmeyer flask. Then, the mixture was heated over a Bunsen burner while stirring with a stirring rod until the solution was evenly mixed and homogeneous. The medium was sterilized in an autoclave at a temperature of 121°C and a pressure of 15 psi for 15 minutes. After that, let the temperature of the medium drop to 45-50°C, then pour 20 mL into each petri dish and let it solidify (Khairani *et al.*, 2024).

The procedure for making Sabouraud Dextrose Broth is similar to that for Sabouraud Dextrose Agar, with the difference being the amount of media used. A total of 3.25 grams of SDB media is dissolved in 50 mL of distilled water. The SDB media is then sterilized using an autoclave, poured into an Erlenmeyer flask, and left to warm (Rafika *et al.*, 2023) .

Rejuvenation and Preparation of *Candida albicans* Fungal Suspension

Candida albicans isolates were taken with a loop wire, then cultured on the surface of SDB media by scratching and incubating at 37°C for 24-48 hours (Song *et al.*, 2024) .

Preparation of Comparison Solution

The positive control used 2% ketoconazole. 20 mg of ketoconazole tablets were finely ground, then weighed using an analytical balance, dissolved in 1 mL of distilled water, and homogenized. The negative control solution used was 1 mL of distilled water without the extract (Khairani *et al.*, 2024) .

Antifungal Activity Test

Candida albicans isolates were taken using a loop wire, then cultured on the surface of SDA media using a streaking technique. Next, the disc paper was taken with tweezers and soaked for 1-2 minutes in several treatments, namely: 50% concentration of purple cabbage extract, 100% concentration of purple cabbage extract, 100% concentration of purple cabbage spray preparation, 50% concentration of purple cabbage spray preparation, negative control, and positive control in the form of 2% ketoconazole. After soaking, the disc paper was placed on the surface of the media in a petri dish.

The petri dishes were then left at room temperature during the pre-incubation stage, then wrapped in parchment paper and incubated at 22–30°C for 24 hours. The test was carried out with six treatment variations and six repetitions in two batches. The inhibition zones formed were measured using a vernier caliper, from end to end across the surface of the paper disc using equation 1.

$$d = \frac{A + B}{2} - C$$

Information
d = diameter of inhibition zone
A = vertical diameter
B = horizontal diameter
C = disc diameter

Data Analysis Techniques

The results of the purple cabbage extract test were analyzed quantitatively using a non-parametric test with the Kruskal-Wallis method. The Kruskal-Wallis test aims to determine whether there are significant differences between three or more groups in one categorical independent variable against one dependent variable (Jamco & Balami, 2022).

Decisions are made based on a significance level of 5%. If the Kruskal-Wallis test results show a $p\text{-value} < 0.05$, then it can be concluded that there is a statistically significant difference between at least two treatment groups. Conversely, if the $p\text{-value} \geq 0.05$, then there is no significant difference between groups, which means that the treatment does not have a significantly different effect on the observed variable. If the Kruskal-Wallis test results show a significant difference,

the analysis can be continued with further testing using the Mann-Whitney U test method in pairs between groups to determine which groups show significant differences (Nurmaizura *et al.*, 2024). Then, it is continued with Bonferroni correction to avoid type I errors when conducting several statistical tests (Saxon, 2015).

Thus, this statistical analysis provides a strong basis for evaluating the effectiveness of each concentration or treatment of purple cabbage extract in the study, as well as assisting in decision making based on objectively obtained data.

RESULTS AND DISCUSSION

Results

The final result of the thick purple cabbage (*Brassica oleracea*) extract was 68 grams of brownish-purple, with a yield of 68%. The purple cabbage extract obtained was then formulated into a spray preparation with various concentrations. This spray is designed as an alternative preparation for oral candidiasis, which is a fungal infection in the oral cavity that is generally caused by *C. albicans*. The use of a spray dosage form was chosen because it is more practical, easy to use, and allows for even distribution of the active substance on the infected oral mucosa.

Purple cabbage extract is known to contain bioactive compounds such as anthocyanins, flavonoids, and vitamin C, which have antioxidant, anti-inflammatory, and antimicrobial activities (Nawaz *et al.*, 2018). These compounds have the potential to inhibit fungal growth and relieve symptoms of oral inflammation. Therefore, a spray formulation based on purple cabbage extract is expected to be a natural alternative therapy for treating oral candidiasis with minimal side effects.

Samples A, B, C, and D exhibit a distinctive brownish-purple color produced by the high anthocyanin content in purple cabbage. Anthocyanins are pigments from the flavonoid group that naturally produce colors ranging from purple, red, to blue, depending on the acidity (pH) of the surrounding environment (Suhartati *et al.*, 2021).

In this study, samples were formulated in four variants with different concentrations: 100% extract, 50% extract, a spray preparation with 100% extract, and a spray preparation with 50% extract. Each formulation differed in the concentration of the active ingredient and the composition of the additional ingredients used. The purpose of creating these various formulations was to evaluate the differences in the antifungal activity effectiveness of each spray preparation. The results of testing the effectiveness of the purple cabbage extract spray preparation on the growth of *C. albicans* are presented in Table 2.

Table 2: Average Inhibition Zone Diameter (mm) of Purple Cabbage Extract Spray.

Sample	Batch	Repetition	Inhibition Zone Diameter	Average	Category
A Extract 100%	1	1	2.15	1.43	Weak
		2	0.35		
		3	1		
	2	4	0		
		5	2.45		
		6	2.65		
B Extract 50%	1	1	3.25	1.32	Weak
		2	3		
		3	0		
	2	4	1.65		
		5	0		
		6	0		

C Spray 100%	1	1	0	5,33	Moderate
		2	10,8		
		3	1,7		
	2	4	8,45		
		5	4,2		
		6	6,85		
D Spray 50%	1	1	14	6,83	Moderate
		2	1,8		
		3	0,8		
	2	4	10,7		
		5	3,95		
		6	9,75		
E Negative control	1	1	0	0	Weak
		2	0		
		3	0		
	2	4	0		
		5	0		
		6	0		
F Positive control	1	1	15,5	15,7	Strong
		2	15,6		
		3	9,7		
	2	4	16,4		
		5	19,3		
		6	17,7		

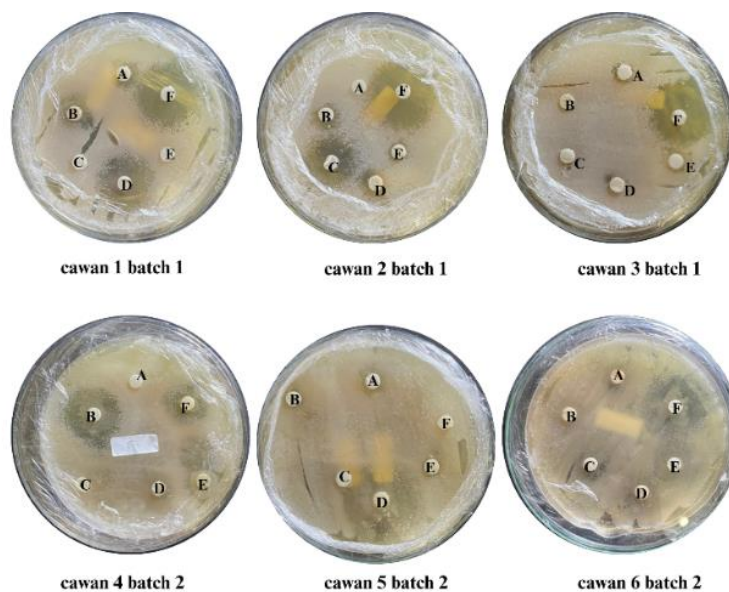


Figure 1: Effect of Purple Cabbage Extract (*Brassica oleracea*) on *Candida albicans*.

Information

A	:	Treatment with 100% purple cabbage extract
B	:	Treatment with 50% purple cabbage extract
C	:	Treatment with 100% purple cabbage extract spray
D	:	Treatment with 50% purple cabbage extract spray
E	:	Negative Control
F	:	Positive Control

DISCUSSION

Measurement of the inhibition zone diameter showed that purple cabbage extract had a significant inhibitory effect on the growth of *C. albicans*. The largest inhibition zone was found in sample D (spray preparation with a concentration of 50%), with an average diameter of 6.83 mm which is included in the medium category. Sample C (spray preparation with a concentration of 100%) also showed moderate antifungal activity, with an average inhibition zone diameter of 5.33 mm. Meanwhile, samples A and B showed weak antifungal activity, with an average inhibition zone diameter of 1.43 mm and 1.32 mm, respectively. These results indicate that the spray dosage form is more effective in inhibiting the growth of *C. albicans* than the direct extract form, possibly due to a more even distribution of the active substance or increased absorption of the active compound in the spray form.

Brassica oleracea extract spray preparation had quite good antifungal activity against *C. albicans*, the main pathogen causing oral candidiasis. This inhibitory activity varied depending on the extract concentration and the composition of additional ingredients in each formulation. The largest inhibition zone was obtained in sample D (spray preparation with 50% extract concentration), with an average inhibition zone diameter of 6.83 mm, which is included in the moderate category. Sample C (100% concentration spray preparation) also showed moderate antifungal activity, with an average inhibition zone of 5.33 mm. This finding is also in line with the research of Nurfatimah *et al.* (2024), who used a propolis-based nanoemulsion spray gel preparation against *C. albicans*. Despite using different ingredients, the results of this study showed that the formulation with the highest extract concentration did not always produce the greatest inhibitory power. This indicates that the effectiveness of inhibition is influenced not only by the extract content, but also by the composition of additional ingredients in the formulation.

In contrast, sample A (100% purple cabbage extract) and sample B (50% purple cabbage extract) produced an average inhibition zone of 1.43 mm and 1.32 mm, respectively. These results are in line with Khairani's (2024) research, which showed that increasing the extract concentration is directly proportional to the increase in the amount of active substances that act as antifungal agents, so that the ability to inhibit the growth of *C. albicans* becomes more effective. Although these two samples are included in the category of weak antifungal activity, there is an average increase in sample A compared to sample B.

Meanwhile, sample E (negative control) did not show any inhibition zone, indicating no antifungal activity and sample F (positive control, 2% ketoconazole) produced an average inhibition zone of 15.7 mm, which is included in the strong category. This shows that ketoconazole has high effectiveness in inhibiting the growth of *C. albicans*. The mechanism of action of ketoconazole is by inhibiting the growth of various types of fungi by suppressing enzyme activity and changing the permeability and function of fungal cell membranes. This disrupts the process of transporting important substances which causes metabolic imbalance and inhibits the synthesis required for fungi (Alfiah *et al.*, 2015).

Measurements that had been carried out were then analyzed using a statistical approach using Kruskal-Wallis, showing a test value of 23.85. This value is higher than the critical value in the T table for a significance level of 5% ($\alpha = 0.05$) and 5 degrees of freedom, which is 11.070. Thus, it can be concluded that there is a statistically significant difference between the six samples on the growth of *C. albicans* (Jamco & Balami, 2022). To identify significant differences in effectiveness between samples, further analysis was carried out using the Mann-Whitney U test. The Mann-Whitney U test is applied as a non-parametric approach for non-normally distributed data. The results of the analysis showed

significant differences in several sample pairs, namely A with E, A with F, B with D, B with F, C with E, C with F, D with E, D with F, and E with F. These findings indicate variations in effectiveness between groups at the initial stage of the analysis.

Considering that the large number of comparisons performed can increase the risk of type I error (*false positive*), the significance level was adjusted using the Bonferroni correction (Saxon, 2015), namely by dividing the alpha value ($\alpha = 0.05$) by the number of comparisons ($n = 15$), so that a new significance threshold of $p < 0.0033$ was obtained. After applying this correction, no sample pairs were found that showed statistically significant differences. However, several pairs, namely AE, AF, BF, CF, DE, and EF, had p values approaching the significance limit ($p = 0.004$), so they can be categorized as approaching significant. Meanwhile, the pairs BD, CE, and DF did not show statistical significance.

Interestingly, sample D (50% purple cabbage extract spray) showed no significant difference compared to the positive control (sample F), indicating that its effectiveness was close to that of the control. While these results cannot be used as proof that both are equally effective, they provide an initial indication that purple cabbage extract at this concentration has the potential to be developed as an antifungal preparation against *Candida albicans*. Therefore, further studies with a more robust research design and larger sample size are needed to confirm these findings.

Research conducted by Ayshwarya and Sudha (2015) showed that purple cabbage extract has significant antimicrobial activity against various pathogenic bacteria. The methanol extract of purple cabbage showed a high level of inhibition against most bacteria, presumably due to the content of bioactive compounds such as alkaloids, tannins, flavonoids, steroids, and quinones. These findings support the hypothesis that active compounds in purple cabbage extract, such as alkaloids, tannins, and flavonoids, contribute to the antimicrobial activity. However, it should be noted that the study focused on bacteria, while in this study, inhibition was also found against the fungus *C. albicans*.

Overall, the results of this study indicate that a purple cabbage extract spray has potential as a natural antifungal agent against *C. albicans*, with optimal effectiveness at a 50% concentration. This finding opens up opportunities for further development as an alternative or complementary treatment for oral candidiasis caused by *C. albicans*. (Kurniawana *et al.*, 2022; Simanullang *et al.*, 2025). Therefore, further research is needed to strengthen these findings and further explore the antifungal potential of purple cabbage extract. The content of secondary metabolites such as flavonoids, saponins, tannins, and steroids is thought to contribute to its antifungal activity. This activity is demonstrated by the formation of inhibition zones in the test medium after paper discs are soaked in purple cabbage extract solutions at certain concentrations (Khairani *et al.*, 2024) .

CONCLUSION

Based on the results of data analysis, it can be concluded that purple cabbage extract (*Brassica oleracea*) formulated in the form of a spray preparation showed a significant effect on the growth of *Candida albicans* fungus as the cause of oral candidiasis. The antifungal activity of this extract was proven by the formation of an inhibition zone at both concentrations of the spray preparation tested. The 50% concentration produced the largest inhibition zone, with an average of 6.83 mm which is classified as a medium category. The results of this study indicate that purple cabbage extract has potential as a natural antifungal agent and can be further developed as an alternative therapy for the treatment of oral candidiasis.

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