



**ANTIFUNGAL ACTIVITY OF THE ETHANOLIC EXTRACT OF WHITE CHRYSANTHEMUM LEAVES (*CHRYSANTHEMUM MORIFOLIUM* (RAMAT.) HEMSL AGAINST *CANDIDA ALBICANS* AND *PITYROSPORUM OVALE***

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**ABSTRACT**

White chrysanthemum (*Chrysanthemum morifolium* (Ramat.) Hemsl.) is an ornamental plant cultivated in Raya Village, Berastagi, North Sumatera which has potential as an antifungal. In this study, the antifungal activity test used agar diffusion method with paper disc as media in various concentrations, namely 500; 250; 50; 10; 5; 2.5; 1.25; 0.625 mg/ml with positive control of ketoconazole and negative control of DMSO. Minimum Inhibitory Concentration (MIC) of ethanol extract of white chrysanthemum leaves against *Candida albicans* and *Pityrosporum ovale* at a concentration of 10 mg/ml with inhibition zone diameters of 6.83 mm and 7.76 mm.

**Keywords:** Antifungal, *Candida albicans*, *Pityrosporum ovale*, white chrysanthemum leaves,

**INTRODUCTION**

The tropical climate and fertile soil in Indonesia allow various types of plants to thrive, both those that are deliberately cultivated and those that grow wild. One of the plants which is quite abundant in Indonesia is the white chrysanthemum (*Chrysanthemum morifolium* (Ramat.) Hemsl.

*Chrysanthemum* leaves have many benefits as antibacterial, antifungal, anti-inflammatory, anti-allergic, anti-rheumatic, relaxing the body, and improving blood circulation (Yulianti, 2019). This is because *chrysanthemum* leaves contain secondary metabolites such as alkaloids, tannins, glycosides, flavonoids, terpenoids/steroids, and saponins (Ujah et al., 2021)

*Candida albicans* is the normal flora of the human body on the skin, digestive tract, respiratory tract and female genital tract.

*Pityrosporum ovale* is a unicellular fungus that grows as normal flora on the scalp. Under normal conditions, the growth rate *Pityrosporum ovale* less than 47%. However, if there is a trigger that can disturb the balance of normal flora on the scalp it can increase the growth rate *Pityrosporum ovale* up to 74% (Utami et al., 2018).

In the research conducted by Sassi et al (2008) it was found that the methanol extract of *chrysanthemum* leaves (*Chrysanthemum grandiflorum*) has antifungal activity against *Candida parapsilosis* with an inhibition zone of 8.4 mm and against *Cryptococcus neoformans* with an inhibition zone 10.6 mm.

Based on the literature which explains that *chrysanthemum* leaves have potential as antifungal, the researchers are interested in testing the antifungal activity of the ethanol extract of white *chrysanthemum* leaves against *Candida albicans* and *Pityrosporum ovale*.

**METHOD**

The sample used in this study was white *chrysanthemum* leaves (*Chrysanthemum morifolium* (Ramat.) Hemsl) whose leaves are fresh as well with plant age of about 3 months or more obtained from Raya Village, Berastagi, North Sumatera.

**Phytochemical Screening and Simplisia Characterization**

Phytochemical screening of ethanol extract of white *chrysanthemum* leaves includes examination compounds belonging to the class of alkaloids, flavonoids, glycosides, saponins, steroids/triterpenoids and tannins.

The simplicia characterization was carried out to determine the quality of the simplicia by comparing the results obtained with data from the literature. The tests carried out were the determination of water content, water soluble extract content, ethanol soluble extract content, total ash content, and acid insoluble ash content in simplicia.

### **Preparation of Ethanol Extract White Chrysanthemum Leaves**

The extract was prepared by maceration, 500 grams of simplicia was put into a glass container, poured with 75 parts of 96% ethanol, left for 5 days protected from light while stirring, filtered and re-macerated for 2 days with 25 parts of ethanol, filtered. Maserate is concentrated by using rotary evaporators at  $\pm 50^{\circ}\text{C}$  until most of the solvent has evaporated and the evaporation process is continued in the oven at  $50^{\circ}\text{C}$  until a thick extract is obtained

### **Antifungal Activity Testing**

The method used to determine the *minimum inhibitory concentration* (MIC) uses the disc diffusion method which was tested triplo. Pour 15 ml of SDA media into a sterile petri dish, let stand until it solidifies. Dipped cotton swab to each inoculum, streaked over the entire surface media. Placed paper discs that had been soaked with various concentrations of the ethanol extract of white chrysanthemum leaves test solution (0.625; 1.25; 2.5; 5; 10; 50; 250; 500 mg/ml), ketoconazole as a positive control and negative control used was DMSO. Then it was incubated at  $25^{\circ}\text{C}$  for 48 hours. The diameter of the inhibition area around the paper holder was measured with a vernier caliper.

## **RESULT AND DISCUSSION**

### **Phytochemical Screening and Simplicia Characterization**

The results of the simplicia phytochemical screening of the ethanol extract of white chrysanthemum leaves contained alkaloids, tannins, saponins, flavonoids, steroids and glycosides.

The water content of the simplicia obtained was 7.32%, which complied with the quality requirements of  $\leq 10\%$ . Determination of the water content is related to the purity of the extract, water content that is too high will cause the growth of microbes which will reduce the stability of the extract (Utami et al, 2017).

Determination of the levels of dissolved compounds in water and ethanol solvents is intended as an estimate of the amount of active compounds that are polar (dissolve in water) and are polar-nonpolar (dissolve in ethanol). Result of ethanol soluble extract content and extract content water soluble obtained were 15.18% and 26.82%. The results obtained showed that white chrysanthemum leaf simplicia was more soluble in water than ethanol and showed that the polar compounds contained in white chrysanthemum leaf simplicia were more than non-polar compounds (Utami et al., 2018).

The purpose of testing the ash content is to provide an overview of the internal and external mineral content originating from the initial process until the extract is formed (Ministry of Health, Republic of Indonesia, 2000). The total ash content obtained was 12.04%, which is quite high. The high ash content indicates the amount of internal mineral content in the white chrysanthemum leaves themselves (Utami et al., 2018).

Acid-insoluble ash content reflects the presence of acid-insoluble mineral or metal contamination in a product. The acid insoluble ash content obtained was 3.15%. The high acid-insoluble ash content indicates the presence of silicate content originating from soil or sand, soil and metallic elements silver, lead and mercury (Utami et al., 2018)

### **Antifungal Activity Test Result**

The results of the antifungal activity test of ethanol extract of white chrysanthemum leaves against *Candida albicans* and *Pityrosporum ovale* showed the presence of inhibition which was indicated by the presence of a clear area around the disc paper which was not overgrown with fungal. The results of measuring the diameter of the inhibition zone of the ethanol extract of white chrysanthemum leaves against *Candida albicans* and *Pityrosporum ovale* can be seen in table 1.

**Table 1. Results of measuring the diameter of the inhibition zone of ethanol extract of white chrysanthemum leaves**

Extract concentration (mg/ml)	Zone of inhibition (mm)	
	<i>Candida albicans</i>	<i>Pityrosporum ovale</i>
500	14.76	16.70
250	13.73	14.43
50	9.23	10.50
10	6.83	7.76
5	-	-
2.5	-	-
1.25	-	-
0.625	-	-
Positive control (Ketoconazole)	14.46	16.46
Negative control (DMSO)	-	-

Table 1. shows that the increase in extract concentration is directly proportional to the diameter of the inhibition zone, that is, the higher the concentration of the extract, the greater the diameter of the inhibition zone formed (Faturrahman et al., 2021).

Minimum Inhibitory Concentration (MIC) of ethanol extract of white chrysanthemum leaves against *Candida albicans* and *Pityrosporum ovale* at a concentration of 10 mg/ml with inhibition zone diameters of 6.83 mm and 7.76 mm.

Based on the criteria of Davis and Stout (1971), the antifungal power of the ethanol extract of white chrysanthemum leaves on *Candida albicans* concentrations of 10 mg/ml and 50 mg/ml are categorized as moderate, at concentrations of 250 mg/ml and 500 mg/ml are categorized as strong. While the antifungal power on *Pityrosporum ovale* concentration of 10 mg/ml is categorized as moderate, at concentrations of 50 mg/ml, 250 mg/ml, and 500 mg/ml are categorized as strong.

The positive control used was ketoconazole with an inhibitory diameter of *Candida albicans* 14.46 mm and *Pityrosporum ovale* 16.46 mm. Ketoconazole was chosen because ketoconazole is an antifungal derivative of imidazole which has a broad spectrum and is fungistatic, this drug produces sufficient plasma levels to suppress the activity of various types of fungi (Ganiswarna, 2016).

The negative control used was DMSO which didn't provide an inhibition zone. This is consistent with the statement that DMSO is a solvent that can dissolve polar and nonpolar compounds and willn't interfere with the observations because it doesn't provide activity

against the growth of fungi (Octaviani et al., 2019).

The formation of the diameter of the inhibition zone due to the administration of white chrysanthemum leaf ethanol extract indicates that there are bioactive compounds that play a role in inhibiting fungal growth *Candida albicans* and *Pityrosporum ovale*.

Flavonoid have antifungal activity with a mechanism of action through the activity of transpeptidase enzymes and lipophilic properties. The transpeptidase enzyme binds to dissolved proteins in the fungal cell wall (mannoprotein layer) causing damage to the cell wall resulting in fungal cell lysis. The lipophilic nature causes damage to the fungal cell membrane (Setiawan et al., 2022).

Alkaloids can damage cell membranes through strong bonds with ergasterol which is the main constituent of fungal cell membranes. Damage to the fungal cell membrane will cause leakage of the cell membrane, as a result of which the essential compounds contained in the fungus come out of the cell, causing the death of the fungus (Marantika & Trimulyono, 2019).

Saponins are surfactants in polar form so that they break down the fatty layer on the cell membrane which in turn causes an increase in the permeability of the cell membrane through a decrease in the surface tension of the sterol membrane on the fungal cell wall, so that metabolic substances in the fungal cells exit the cell and result in death (Hidana & Fauziyyah, 2016).

Tannin mechanism is by causing cell membrane irregularities by changing membrane permeability especially in the process of transporting essential substances, causing

metabolic imbalances so that the fungus experiences death (Hidana & Fauziyyah, 2016).

Steroids are lipophilic and have the potential as antifungals by inhibiting the formation of ergosterol (Lutfiyanti et al., 2012).

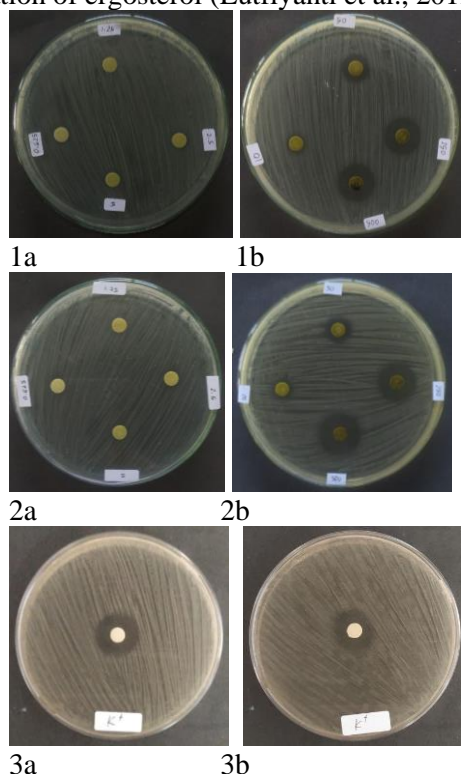


Fig 1 : Zone of inhibition against *Candida albicans* (a:concentration 0.625; 1.25; 2.5; 5 mg/ml and b: concentration 10; 50; 250; 500 mg/ml).

Fig 2 : Zone of inhibition against *Pityrosporum ovale* (a:concentration 0.625; 1.25; 2.5; 5 mg/ml and b: concentration 10; 50; 250; 500 mg/ml).

Fig 3 : Zone of inhibition of positif control ketoconazole (a:against *Candida albicans* and b: against *Pityrosporum ovale*).

## CONCLUSION

Based on the results of the research that has been done, it can be concluded that: Simplicia and ethanol extract of white chrysanthemum leaves contain alkaloids, tannins, saponins, flavonoids, steroids and glycosides. Minimum Inhibitory Concentration (MIC) of ethanol extract of white chrysanthemum leaves against *Candida albicans* and *Pityrosporum ovale* at a concentration of 10 mg/ml with inhibition zone diameters of 6.83 mm and 7.76 mm

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