Inhibitory activity of Candida albicans fungi by acetone extract of the lichen Usnea sp.

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Inhibitory Activity of *Candida albicans* Fungi by Acetone Extract of the Lichen *Usnea* sp.

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Abstract. Lichen is a unique organism that produces various organic compounds such as primary and secondary metabolites. In this study, we focused on reporting the growth inhibitory activity of *Candida albicans* fungi by acetone extract of the lichen *Usnea* sp. The *Usnea* sp. powder was extracted by the maceration method for 3x24 hours using acetone solvent. The results of phytochemical screening showed that the extract contained a class of chemical compounds such as alkaloids, saponins, tannins, and flavonoids. Bioactivity test against inhibition growth of *C. albicans* fungi at concentrations of 1.25%, 2.5% and 5% were 16.0 cm, 32.5 cm and 39.0 cm, respectively. The results of this study are a very important report to the exploration of natural product compounds to treat the candidiasis disease caused by the *C. albicans* fungi.

INTRODUCTION

Fungal infections are common in tropical countries due to hot and humid climates to facilitate the growth of fungi. As a solution, many antifungal drugs have been used which are resistant to certain fungi. As a solution, many chemical antifungal drugs have been used that are resistant to certain fungi. *Candida albicans* is one of the commensal organisms that cause diseases in the human body, such as Candidiasis [1]. The need for antifungal drugs that have strong bioactivity is increasingly emphasized as many reports of fungal infections harm humans [2].

The use of lichen as an antibiotic has traditionally been carried out for a long time, thus attracting the attention of scientists. The properties of this antibiotic include antibacterial, antifungal, and antiviral. Lichens produce secondary metabolites belonging to many classes, including amino acid derivatives, peptides, pulvinic acids, terpenoids, carotenoids, aliphatic acids, steroids, monocyclic phenols, depsides, dibenzofurans, anthraquinones, xanthones, usnic acid, and other compounds [3]. Lichen habitats can be found in tree trunks, rocks, soil, walls, and other substrates with various environmental conditions, both in the desert and polar regions [4,5].

Usnea sp. is one of the lichen species belonging to the *Usneaceae*. *Usnea* sp. is found in mountainous areas in Indonesia, India, China, Japan, Africa, Malaysia, Australia, America, New Zealand, England, and Central America [6]. Several types of lichen from the genus *Usnea* sp. that have been widely used as traditional medicine include species *U. articulate, U. dasypoga, U. longissima, U. sternaii, U. vrieseana, U. comosa, U. blepharea, U. bayleyi, U. javanica and U. flexuosa* [7,8]. In Indonesia, *Usnea* sp. can be found in almost all mountains with an altitude ranging from 1000 m above sea level.

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Several previous studies have reported various biological activities of *Usnea* sp. very interesting, for example as an antiproliferative [9], antibiotics [10], antifungal [11], anticancer [12], antimalarial [13,14], antibacterial [15,16], antidiabetic[17], and antioxidants [18,19]. Based on the description above, it provides a basis for conducting research that can be carried out on other lichen species to test their bioactivity. This research will study the *Usnea* sp. originating from Sulawesi. In this study, we focused on reporting the growth inhibitory activity of *C. albicans* fungi by acetone extract of the lichen *Usnea* sp.

MATERIAL AND METHODS

Materials

The materials used were *Usnea* sp. collected from Latimojong village mountains, Enrekang district, South Sulawesi Indonesia. The reagents were 70% acetone (Sigma-Aldrich), hydrochloric acid (Sigma-Aldrich), ethanol (Merck), $FeCl_3$ (Sigma-Aldrich), NaCl (Sigma-Aldrich), DMSO (Merck), aquadest (One-Lab Water One), Potato Dextrose Agar medium (Oxoid), ketoconazole (Tab Dexa), *a* microbial culture of *C. albicans* and Whatman 42 filter paper.

Extraction

The *Usnea* sp. that has been successfully collected is cleaned of impurities. *Usnea* sp. is crushed to form a powder. 700 g of *Usnea* sp. powder was extracted by maceration using 70% acetone as solvent. *Usnea* sp. was soaked in 70% acetone for 3x24 hours and filtered every 1x24 hours. The residue obtained was soaked again with a new solvent of 70% acetone. The filtrate from each filter is combined and evaporated to separate the solvent using an evaporator.

Screening Phytochemical

The acetone extract was tested for the class of secondary metabolites contained, in it by taking a small sample from the macerated extract and adding reagents according to the compound to be identified. This work refers to the research of Maulidiyah et al. [20].

Antifungal Test

The process of testing the growth inhibition of *C. albicans* was carried out under aseptic conditions. The test was carried out using the disc diffusion method, the diameter of the paper disc made was 5 mm. The series variation of the concentration of acetone extract was made into 1.25%, 2.5%, and 5% which were tested on *C. albicans* with a density of 1.4x106/mL. Determination of the activity of acetone extract against *C. albicans* was carried out by calculating the diameter inhibition zone or the clear zone formed around the paper disc.

RESULTS AND DISCUSSION

Extraction

The maceration technique has several advantages is not need heating, so it is less likely that natural materials will be damaged or decomposed. The choice of acetone as a solvent is based on the ability of the solvent to attract polar and non-polar compounds contained in the sample. Phytochemical screening is useful for knowing the class of compounds contained in the acetone extract of *Usnea* sp. The results of the phytochemical screening of the extract showed that it contained a group of chemical compounds such as alkaloids, saponins, tannins, and flavonoids, as shown in Table 1. This is similar to a previous study that reported phytochemical screening of extracts of *Usnea* sp. [21].

TABLE 1. Phytochemical screening of the acetone extract for *Usnea* sp.

Sample	Compound Content				
	Alkaloid	Terpenoid	Flavonoid	Tannin	Saponin
Usnea sp. extract	+	-	+	+	+

Antifungal Test

There were three groups of test materials, namely acetone extract with varying concentrations of 1.5%, 2.5%, and 5% and ketoconazole as a positive control and DMSO as a negative control. The antifungal activity of *Usnea* sp. showed a clear zone formed in the test area of the fungus *C. albicans*, where the activity of the diameter of the inhibition zone (DDH) was 16 mm at a concentration of 1.25% (strong). The test results showed that there was growth inhibition activity of *C. albicans* by acetone extract indicated by the presence of a clear zone formed around the paper disc (Fig. 1). At each concentration of the extract showed very strong effectiveness based on the table of inhibition categories [22]. The increase in the concentration of the extract showed that the inhibitory effect was also getting better as shown in Table 2. In the positive control of ketoconazole 2%, DDH was formed by 29.5 mm (very strong) and in the negative control, DMSO did not have an inhibition zone.

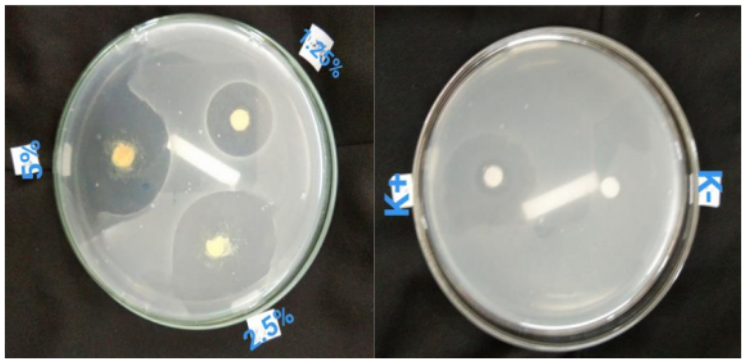

FIGURE 1. Inhibition zone of acetone extract against the growth of the fungus *C. albicans*

TABLE 2. Table of antifungal activity test

Test sample	Concentration (%)	Inhibition Zone Diameter (mm)	Inhibition Category
Usnea sp. extract	1.25	16	Very strong
	2.5	32.5	Very strong
	5	39	Very strong
Positive control (Ketokonazole)	2	29.5	Very strong
Negative control (acetone)	10	0	Not active

According to [22], compounds that have antifungal activity include alkaloids, flavonoids, steroids, polyphenols, and tannins. The active metabolites can damage mitochondrial membranes and cristae, which causes a decrease in O_2 uptake; this results in reduced ATP energy produced for the process of cell growth and development so that the growth of the fungus *C. albicans* is normally inhibited [23,24]. In addition, antifungal compounds actually have various inhibitory mechanisms against fungal cells. Antifungal compounds have a mechanism of action by neutralizing enzymes involved in fungal invasion and colonization, damaging fungal cell membranes, inhibiting fungal enzyme systems so that they interfere with the formation of hyphae tips and affect nucleic acid and protein synthesis [16].

CONCLUSION

Acetone extract of *Usnea* sp. contains active compounds, namely alkaloids, saponins, tannins, and flavonoids. These secondary metabolites act as antifungals. Bioactivity test against inhibition growth of *C. albicans* fungi at concentrations of 1.25%, 2.5% and 5% were 16.0 cm, 32.5 cm and 39.0 cm, respectively.

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