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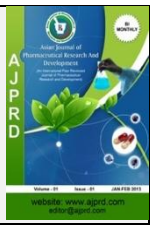

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Review Article

Test of Active Compound and Activity Pirdot Leaf Extract (*Sauraia Vulcani Korth.*) on *Candida Albicans* Growth in Vitro

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A B S T R A C T

Objective: to determine the activity of pirdot leaf extract on the growth of Candida albicans in vitro

Design: this study was a true experiment with a post-test only control group research design. The method used agar diffusion using Kirby Bauer technique. This study used 4 variations concentration namely 10%, 20%, 40%, and 80%, positive control (ketoconazole tablets), and negative control (Dimethyl Sulfoxide). The data obtained were analyzed using descriptive statistical analysis.

Interventions: the intervened variable were compound content and antifungal activity of pirdot leaf extract concentration of inhibitory effect.

Main outcome measures: the main measurement in this study was the inhibition concentration of pirdot leaf extract on *Candida albicans* growth

Results: the results showed that the inhibitory from a concentration of 10% of 8.96 mm, the highest inhibition was at 80% of 18.33 mm.

Conclusion: It could be concluded that pirdot leaf extract has antifungal activity against *Candida albicans.*

Keywords: *Antifungal; Candida albicans; Diffusison Method; Saurauia vulcani Korth*

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INTRODUCTION

Fungal infections are of particular concern in countries with tropical climates. Indonesia with a tropical climate is caused by humid air and inadequate sanitation. Indonesia has a densely populated environment and a low socio-economic level. The fungus Candida albicans is considered a pathogenic species and one of the highest causes of infection compared to other fungi. This fungus is the main cause of candidiasis. Candida albicans species are opportunistic fungi that cause thrush, skin lesions, vulvavaginistik, candida in the urine (candiduria), gastrointestinal candidiasis which can cause gastric ulcers, or can even complicate cancer[1].

Pharmacological candidiasis treatment is carried out either orally or intravaginally using azole class antibiotics[2]. However, an unwanted side effect that commonly occurs in therapy with the azole class is gastrointestinal disturbances and can cause abnormalities in liver enzymes[3]. This treatment can be selected as an alternative. Along with the development of increasingly sophisticated technology, the use and utilization of traditional medicines in Indonesia has increased dramatically. Traditional medicines are being used again by people who believe in the principle of back to nature as an alternative treatment, in addition to synthetic medicines that are developing in the market[4,5].

Traditional medicines derived from plants and pure natural ingredients have side effects, levels of danger and risk that are much lower than chemical drugs[6]. In addition, the use

of traditional medicine is generally chosen because of the cost factor and the inconvenience of a medical evaluation. Traditional medicines commonly used to treat vaginal discharge by Indonesians include betel leaf, beluntas and jawer kotok[7].

Medicinal plants have the potential to be used as natural fungicides. This is because medicinal plants contain secondary metabolite compounds which can act as antifungals. Secondary metabolites such as saponins, alkaloids, coumarin, xanthone, flavonoids, fatty acids, phenolic compounds, terpenes, essential oils, lectins and polypeptides have been reported to have antifungal activity[8,9,10].

Previous research on the leaves of this pirdot plant said that this plant had been used by the people of North Sumatra for a long time to treat wounds and prevent bacterial infections. Pirdot leaves contain compounds in the form of steroids, flavonoids, saponins, tannins, triterpenes, and also have antioxidant power. Secondary metabolites found in pirdot leaves are thought to have the potential to be antifungal, so testing is needed to update the potency of pirdot leaves[11,12,13]. So, the researchers conducted an antifungal test of the ethanol extract of pirdot against Candida albicans.

MATERIALS AND METHODS

Materials

The materials used were pirdot leaves (*Sauraia vulcani* Korth.) Obtained from Parapat, Simalungun Regency, *Candida albicans* cultures obtained from the Microbiology Laboratory of Grandmed Lubuk Pakam Hospital, North Sumatra, Potato Dextrose Agar (Oxoid) medium, 96% ethanol (Merck), ketoconazole tablets, Dimethyl Sulfoxide (DMSO) (Merck), distilled water, alpha naphthol (Merck), amyl alcohol (Merck), acetic anhydridic acid (Merck), concentrated hydrochloric acid (Merck), concentrated nitric acid (Merck), acid concentrated sulfate (Merck), iron (III) chloride (Merck), bismuth nitrate, potassium iodide (Merck), chloroform (Merck), sodium hydroxide (Merck), chloralhydrate (Merck), magnesium powder (Merck), iodine, mercury (II) chloride (Merck), lead (II) acetate (Merck) and toluene (Merck).

Plant extract preparation

The sample was collected purposively, that is, without comparing it with the same sample from other areas obtained from Parapat, Simalungun Regency. The part of the plant taken were the old leaves. The leaves are cleaned of dirt and then dried. Preparation ethanol extract of pirdot leaves (EEPL) by maceration method with 96% ethanol solvent. A total of 500 grams of pirdot leaf simplicia powder is put into a closed vessel then 5 liters of 96% ethanol are added and left for 5 days protected from light while frequently stirring. Then filtered, the results of the filter or liquid filtrate of pirdot leaf ethanol extract are evaporated using a rotary evaporator (Heidolph, Germany), then re-evaporated using a waterbath (Memmert, Germany) to ensure that all the solvent has evaporated to get a thick extract of pirdot leaves.

Extract characterization

Examination of secondary metabolite content by phytochemical screening against simplicia and EDP powders, includes examination of glycosides, flavonoids, saponins, tannins and steroids[14].

Analysis of the Ethanol Extract Compound of Pirdot leaves by Thin Layer Chromatography method

Phytochemical screening examination on the ethanol extract of pirdot leaves was carried out by the Thin Layer Chromatography (TLC) method. A total of 100 mg of the extract were dissolved in 1 ml of each extract solvent and then dotted in the stationary phase. The stationary phase used was a plate coated with silica gel 60 F254 (Merck, Germany) measuring 10x5 cm. Then the plate is inserted into the chamber which is saturated with mobile phase steam. The motion phase used is in accordance with the examination being carried out. After the development is complete, the plate is removed and dried, then the plate is sprayed with the appearance of the spots and heated in an oven at 110^{o}C for 5 minutes then the color change were observed.

Inoculum preparations

Fungal colonies were taken from the culture stock with sterile loop needles and then suspended in a test tube containing 5 ml of 0.9% NaCl solution[15]. Tfhen the turbidity of the solution was measured at a wavelength of 520 nm until 25% transmittance was obtained[16].

Preparation of extract test solutions with various concentrations

Preparation of the pirdot leaf extract sample solution was made in 4 concentrations: 10%, 20%, 40%, and 80% (g / ml). The test concentration was made by weighing the extract respectively 0.10g, 0.2g, 0.4g, and 0.8 g with analytical scales, then each dissolved with 1 ml of DMSO (Dimethyl Sulfoxide) as much as 1 ml[17].

Antifungal Activity Test

The inoculum of *Candida albicans* was put into 6 sterile petri dishes, then 10 ml of Potato Dextrose Agar (PDA) was poured. The petri dish is then shaken on the table surface (Laminar Air Flow Cabinet) so that the media and the mushroom suspension are mixed and then left for a few minutes until solidified. Antifungal activity was tested using the agar diffusion method (Kirby Bauer) using a paper holder. Put a paper holder that has been soaked for 30 minutes with a test solution of pirdot leaf ethanol extract concentrations of 10%, 20%, 40%, and 80%, positive control (ketoconazole tablets), and negative control (Dimethyl Sulfoxide) on the surface of the media to make it solid fungi that have been inoculated and left for 15 minutes, then incubated in an incubator at 37 ° C for 24 hours. After that, measuring the diameter of the inhibition area around the paper cutter using a caliper was carried out three times[15].

RESULT AND DISCUSSION

Determination of chemical compounds for simplicia and ethanol extract of pirdot leaves (EEPL) was carried out to obtain information on groups of secondary metabolite compounds contained therein. The examination carried out on simplicia and extracts is an examination of the class of alkaloid compounds, flavonoids, tannins, saponins, glycosides and steroids / triterpenoids. The results of phytochemical screening for simplicia and EEPL powders can be seen in Table 1. It showed that simplicia and EDP contain the same chemical compounds, namely alkaloids, flavonoids, tannins, saponins, glycosides[14].

Table: 1. Phytochemical screening results of simplicia powder and ethanol extract of pirdot leaves

No	Screening	Simplicia	Extract
1	Alcaloids	+	+
2	Flavonoids	+	+
3	Tannins	+	+
4	Saponins	+	+
5	Glycosides	+	+
6	Steroids/triterpenoids	-	-

Identification of flavonoids using TLC used the mobile phase of Chloroform: Ethyl Acetate (7: 3) and a black stain appeared after spraying with 10% $FeCl_3$. From the results of TLC in Table 4.2, it can be seen that there are black stains on the ethanol extract with Rf values of 0.64, 0.70, 0.86 and 0.87 which confirms the content of the flavonoid quercetin compound in chloroform: ethyl acetate. The Rf value of the pirdot leaves extract can be seen in Table 2.

Table: 2. Rf value and stain color from phytochemical screening of ethanol extract of pirdot leaves

No	Flavonoid examination	Ratio	Rf	Quercetine
1	Chloroform: Ethyl Acetate	7:3	R_f1=0.64 R_f2=0.70 R_f3=0.86 R_f4=0.87	R_f1=0.63
2	Chloroform: Ethyl Acetate	8:2	R_f1=0.67 R_f2=0.84	R_f1=0.65

Based on table 2, it could be seen that the quercetin standard has an Rf value of 0.63 and is blackish brown. In the pirdot extract samples had the same color and the Rf value of the Rf extract was 0.64 close to the quercetin standard. This indicates that the extract sample contains one type of flavonoid, namely quercetin.

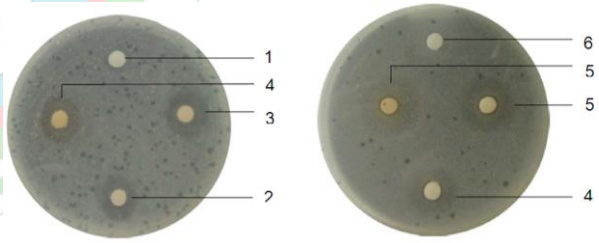
The greater the concentration of the extract given, the greater the inhibition that is formed, because the more concentration of bioactive components contained in the extract. The effectiveness of an antifungal agent is influenced by the concentration of the substance given. Increasing the concentration of the extract results in a high content of active ingredients that function as antifungal so that the ability to kill fungal growth is also greater[18]. In this study, ketoconazole was used as a positive control and DMSO as a negative control[19]. The positive control serves to compare the inhibitory power of pirdot leaf extract with chemical drugs that are proven and often used as an antifungal, while the negative control is used to determine whether the solvent used can affect the results of the antifungal test or not[19]. The inhibition ability test of pirdot leaf extract on the growth of *Candida albicans* fungi was carried out using 4 concentrations, starting from a concentration of 10%, 20%, 40%, and 80% and using a positive control comparison of ketoconazole and negative control with sterile DMSO solvent. The results of the pirdot leaf extract inhibition test on the growth of *C. albicans* can be seen in Table 3.

Table: 3 Average of extract inhibition against fungal growth

No	Extract concentration	Average of extract inhibition against fungal growth (mm)
1	EEPL 10%	8.96
2	EEPL 20%	11.23
3	EEPL 40%	13.50
4	EEPL 80%	18.33
5	Positive control	21.2
6	Negative control	0

Notes: EEPL (Ethanol Extract of Pirdot Leaves)

Observation of the antifungal activity of pirdot leaf extract against Candida albicans was carried out for 24 hours. The parameter measured is the diameter of the inhibition zone formed on the edge of the disc paper area. Measurement of the formed zone of inhibition is done by measuring the diameter of the clear zone formed using a caliper. After that the formed inhibition zone was compared with the inhibition zone diameter of the ketoconazole positive control zone.



Fingure: 1. The results of measurements of the inhibition zone of pirdot leaf ethanol extract against the fungus Candida albicans at several concentrations.

Notes: 1. EDP 10% 4. EDP 80%
2. EDP 20% 5. Positive Control
3. EDP 40% 6. Negative Control

The results of the measurement of the inhibition area in Table 2 and Figure 1 show that the greater the concentration of the extract given will produce a larger inhibition area, this is due to the more active substances contained in the extract. The minimum inhibitory concentration (MIC) of pirdot leaf extract was at a concentration of 10%, namely 8.96 mm and the diameter of the largest inhibition area was at a concentration of 80%, namely 18.33 mm. EDP of 10%, 20%, 40%, 80% showed antifungal activity which was not significantly different from the positive control ($p > 0.05$). This shows that the increase in the concentration of pirdot leaves extracts affects the diameter of the inhibition zone formed, the different diameter of the inhibition zone indicates the

ability of different extracts to inhibit the growth of the tested fungi[20,21]. The antifungal activity of ethanol extract and sapodilla fruit skin fraction was caused by the presence of chemical compounds in the form of flavonoids, glycosides, tannins and steroids / triterpenoids. Flavonoid compounds have antifungal activity because flavonoids are a group of phenolic compounds[22,23]. Tannins are included in the polyphenolic compound group so that they have antifungal activity. Phenolic compounds and their derivatives such as flavonoids and tannins are antifungal agents that work by disrupting the function of the cytoplasmic membrane[24].

The difference in the diameter of the inhibition zone can be due to differences in the content of secondary metabolites contained in the extract. This is in accordance with Prescott's opinion which states that the size of the inhibition zone is influenced by differences in the size of the extract concentration. Other factors that influence the differences in the inhibition zone are incubation temperature, disc installation time and the distance of the antimicrobial discs[25].

CONCLUSION

The results of the phytochemical screening test for pirdot leaf extract (*Sauraia vulcani* Korth.) Contained alkaloids, flavonoids, tannins, saponins, and glycosides. Flavonoids, tannins, and saponins are proven compounds that have antifungal properties. The ethanol extract of pirdot also contains flavonoid compounds, namely quercetin. The pirdot leaf extract showed activity against the growth of the fungus *Candida albicans*. The results of the data processing of the minimum inhibitory concentration of pirdot leaf extract on the growth of C. albicans showed that the lowest concentration of 10% concentration was seen to inhibit the growth activity of *C. albicans*.

CONFLICT OF INTERESTS

All author have no to declare

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