Indonesian Journal of Pharmaceutical and Clinical Research
Journal homepage: https://idjpcr.usu.ac.id

ANTIFUNGAL OF SOKA FLOWER (*Ixora Chinensis* Lam.) ETHANOL EXTRACT AGAINST *Candida albicans* AND *Pityrosporum ovale*

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ARTICLE INFO

Article history:
Received 03 Februari 2025
Revised 16 Juni 2025
Accepted 18 Juni 2025
Available online 30 Juni 2025

E-ISSN: 2620-3731
P-ISSN: 2615-6199

How to cite:
Keliat, J. M., & Sumayyah, T. (2025). Antifungal of soka flower (*Ixora chinensis* Lam.) ethanol extract against *Candida albicans* and *Pityrosporum ovale*. *Indonesian Journal of Pharmaceutical and Clinical Research*, *8*(2), 15–24.

ABSTRACT

Pathogenic fungi are microorganisms that cause infectious diseases in humans and are capable of growing and developing in various environments. The types of fungi that most often cause skin infections are *Candida albicans and Pityrosporum ovale*. The soka flower plant (Ixora chinensis Lam.), commonly found in Indonesia, is known to have antibacterial properties. The antifungal strength of the soka flower comes from the natural chemicals in its ethanol extract, which stops the growth of *Candida albicans and Pityrosporum ovale*. The research method was conducted experimentally, which included stages such as the preparation of dry powder, characterization of dry powder, phytochemical screening both on dry powder and the ethanol extract of soka flowers, preparation of the ethanol extract, standardization of the extract, and evaluation of the antifungal activity of the ethanol extract against both types of fungi. The research results indicate that the minimum inhibitory concentration of the ethanol extract of the soka flower against *Candida albicans* is 5.5 mg/ml with an inhibition zone diameter of 8.8 mm ±0.05, while for *Pityrosporum ovale*, the minimum inhibitory concentration is 50 mg/ml with an inhibition zone diameter of 10.7 mm ±0.50. The statistical analysis from Kruskal-Wallis tests show a significant difference in the amounts of ethanol extract from soka flowers based on the size of the inhibition zones for *Candida albicans* and *Pityrosporum ovale*, with probabilities of 0.000 and 0.001, both of which are below 0.05.

Keywords: antifungal; *Candida albicans*; *Ixora chinensis* Lam; *Pityrosporum ovale*

ABSTRAK

Jamur pathogen merupakan mikroorganisme yang menimbulkan penyakit menular pada manusia serta mampu tumbuh dan berkembang di berbagai lingkungan. Jenis jamur yang paling sering menyebabkan infeksi pada kulit adalah *Candida albicans* dan *Pityrosporum ovale*. Tanaman bunga soka (*Ixora chinensis Lam.*), yang banyak dijumpai di Indonesia, dikenal memiliki sifat antibakteri. Kemampuan antijamur bunga soka ini berasal dari kandungan senyawa fitokimia atau metabolit sekundernya yang terdapat dalam ekstrak etanol tanaman tersebut. Penelitian ini bertujuan untuk menilai efektivitas ekstrak etanol bunga soka (*Ixora chinensis Lam.*) dalam menghambat pertumbuhan *Candida albicans* dan *Pityrosporum ovale*. Metode penelitian dilakukan secara eksperimental, yang mencakup tahapan seperti pembuatan simplisia, karakterisasi simplisia, skrining fitokimia baik pada simplisia maupun ekstrak etanol bunga soka, pembuatan ekstrak etanol, standarisasi ekstrak, serta evaluasi aktivitas antijamur dari ekstrak etanol terhadap kedua jenis jamur tersebut. Hasil penelitian menunjukkan bahwa konsentrasi hambat minimun ekstrak etanol bunga soka terhadap *Candida albicans* adalah 5,5 mg/ml dengan diameter zona hambat sebesar 8,8 mm ±0,05 sedangkan untuk *Pityrosporum ovale* konsentrasi hambat minimum adalah 50 mg/ml dengan diameter zona hambat 10,7 mm ±0,50. Analisis statistik dari uji Kruskal-Wallis menunjukkan perbedaan signifikan dalam konsentrasi ekstrak etanol dari bunga soka berdasarkan ukuran zona hambatan untuk *Candida albicans* dan *Pityrosporum ovale*, dengan probabilitas masing-masing 0.000 dan 0.001, yang keduanya di bawah 0.05.

Keyword: anti jamur; *Candida albicans; Ixora chinensis* Lam; *Pityrosporum ovale*

http://doi.org/10.32734/idjpcr.v8i01.19926

1. Introduction

Fungal infections represent a considerable risk to human health, leading to around 1.5 million fatalities globally each year. Fungal infections represent a prevalent issue, impacting approximately 20-25% of the

global population. Since 1980, there has been a notable rise in fungal infections across diverse patient groups [1]. About 25%, equating to 1.7 billion people globally, are affected by superficial fungal infections of the skin and nails [2]. Superficial infections are mainly attributed to various species of *Candida*, recognized as the most prevalent fungal pathogens worldwide [3].

Candida albicans serves as a pathogen responsible for opportunistic and nosocomial infections. Candida albicans ranks among the top five pathogenic organisms linked to sepsis and infections of mucosal surfaces within the gastrointestinal tract. *Candida* can cause serious infections that spread throughout the body, such as candidemia, disseminated candidiasis, endocarditis, meningitis, endophthalmitis, and infections in different internal organs [1].

In addition to *Candida*, another notable fungal infection is *Pityrosporum ovale.* This fungus is a component of the normal skin microbiome; however, it has the potential to become pathogenic under specific circumstances. This species has the potential to cause conditions including scalp and neck dermatitis, seborrheic dermatitis, pityriasis versicolor, and *Malassezia folliculitis*. The occurrence of seborrheic dermatitis notably rises in specific patient subgroups, especially in individuals with human immunodeficiency virus (HIV), where it correlates with reduced CD4 cell counts, as well as in neurological patients, such as those affected by Parkinson's disease and spinal cord injuries [4].

The standard approach to treating fungal infections involves the use of antifungal medications. These include azoles like ketoconazole, miconazole, and imidazole; polyenes such as nystatin, pimaricin, and amphotericin B; allylamines including naftifine and terbinafine; morpholines like amorolfine; and antimetabolites such as 5-fluorouracil. The unregulated application of antibiotics promotes the emergence of multidrug-resistant fungal strains [1].

A potential approach to tackle the challenge of antifungal resistance involves the advancement of traditional remedies by harnessing the properties of plants and their bioactive constituents. The soka flower demonstrates a capacity to eliminate fungi and inhibit the development of resistant cases. Investigations into phytochemical screening tests indicate that the ethanol extract of soka flowers (*Ixora chinensis Lam*.) is composed of glycosides, steroids, flavonoids, triterpenoids, and alkaloids, all of which exhibit antifungal properties [5].

According to the description provided, it is essential to carry out an investigation to evaluate the antifungal properties of the ethanol extract derived from soka flowers. This investigation encompasses the processes of preparing dry samples, characterizing dry samples, conducting phytochemical screening of both dry samples and the ethanol extract of soka flowers, preparing the ethanol extract of soka flowers, standardizing the ethanol extract of soka flowers, and evaluating the antifungal activity of the ethanol extract of soka flowers against *Candida albicans* and *Pityrosporum ovale.* The assessment of antifungal activity is quantified by the minimum inhibitory concentration within the inhibition zone, determined by examining the clear area surrounding the disc paper.

2. Methods

2.1. Chemicals

Acetic acid anhydride, alpha-naphthol, amylalcohol, bismuth (III), bouchardat's reagent, chloralhydrate, chloroform, concentratedhydrochloric acid, concentrated nitric acid, concentrated sulfuric acid, distilled water, DMSO, Dragendorff's reagent, Ethanol 96%, iodine, iron (III) chloride, Isopropanol, Ketoconazole, lead (II) acetate, magnesium powder, mayer's reagent, mercury (II) chloride, methanol, molisch's reagent, n-hexane, potassium iodide, sabouraud dextrose agar (Millipore), sabouraud dextrose broth (Himedia) and Toluene.

2.2. *Preparation of Dry Sample of Soka Flower*

Fresh soka flowers are cleaned of dirt by washing them with running water until they are clean and then drained. We then weigh them using their wet weight. The soft-shelled flowers weighed 3.08 kg. Furthermore, the soka flowers are dried in the refrigerator at a temperature of 30-40°C. Samples are considered dry if they are brittle (crushed into pieces); then the dry sample is sorted to separate foreign objects, then crushed using a blender until smooth to obtain dry powder, then sieved using a sieve with a 60 mesh to form powder. Subsequently, it is stored in a sealed, dry container.

2.3 Characterization of Dry Sample of Soka Flower

2.3.1. Macroscopic Characterization

Macroscopic characterization is carried out by using the five senses to observe the color, shape, smell and taste of fresh plant parts.

2.3.2. Microscopic Characterization

In the microscopic characterization, water reagent,phloroglucin reagent solution, and chloralhydrate reagent solution were used.

2.3.3. Determination of Water Content

Five grams of soft-weighed soka flower powder were placed in a round-bottom flask containing saturated toluene and carefully heated for fifteen minutes. After boiling the toluene, the drip rate was adjusted to roughly two drips per second until the distillation phase began. The interior of the cooler has been cleaned with toluene. The distillation process lasted 5 minutes, after which the receiving tube was allowed to cool to ambient temperature. Once the water and toluene have completely separated, the volume of water is calculated using the water content of the sample under study. The water content is calculated as a percentage.

2.3.4. Determination of Water Soluble Extract Content

Five grams of dry material were macerated for 24 hours in 100 mL of water and 2.5 mL of chloroform in a corked bottle, shaken intermittently for the first 6 hours, and then left to stand for 18 hours before filtration. A warmed and tared flat-bottomed cup was used to evaporate up to 20 mL of the filtrate until it was dry. The residue was heated in a 105°C oven until it reached a steady weight. The desiccated substance has its water-soluble essence content determined.

2.3.5. Determination of Soluble Juice Content in Ethanol

As much as 5 g of dry powder was macerated in 100 ml of 96% ethanol for 24 hours using a blocked bottle, shaking occasionally for the first 6 hours, then left for another 18 hours before filtering. Up to 20 mL of the filtrate was evaporated until dry in a heated and tarred flat-bottomed cup. The residue was baked in an oven at 105°C until a uniform weight was obtained. The dried material has its water-soluble essence content computed.

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2.3.6. Determination of Total Ash Content

Five grams of dried soka flower powder should be accurately transferred to a preheated and tared porcelain container, and then compressed. The porcelain exchanger and its contents are gradually heated until the charcoal is exhausted, then cooled and weighed until a consistent weight is achieved. The dried material's ash content is measured.

2.3.7. Determination of Acid Insoluble Ash Content

Boil the ash used to determine total ash content for 5 minutes in 25 ml of mild hydrochloric acid, then collect the insoluble residue and filter it through ash-free filter paper after rinsing it with hot water. The residue and filter paper were burned until a consistent weight was reached, then cooled and weighed.

2.4. Phytochemical Screening of Soka Flower Powder

2.4.1. Alkaloid Test

0.5 grams of dry powder was measured, followed by the addition of 1 ml of 2 N hydrochloric acid and 9 ml of distilled water. The mixture was heated in a water bath for 2 minutes, then cooled and filtered. The filtrate is utilized for alkaloid testing. Three test tubes were selected, and 0.5 ml of the filtrate was added to each tube. Two drops of Bouchardat, Dragendorff, and Mayer reagents were added to each tube. We classify alkaloids as positive if we observe sediment or turbidity in at least two test tubes from the aforementioned samples.

2.4.2. Flavonoid Test

A total of 10 grams of dry powder was mixed with 10 ml of hot water, boiled for 5 minutes, and filtered hot. Mix 0.1 gramme magnesium powder, 1 ml strong hydrochloric acid, and 2 ml amyl alcohol into 5 ml of filtrate, then set aside to separate. A red, yellow, or orange colour in the amyl alcohol layer suggests the presence of flavonoids. The procedure was repeated for each dry powder.

2.4.3. Glycoside Test

The dry powder was weighed at 3 grams and extracted with 30 ml of a mixture containing 7 parts by volume of 96% ethanol and 3 parts by volume of distilled water, to which 10 ml of 2N hydrochloric acid was added. Refluxed for 10 minutes, then cooled and filtered. Measure 20 ml of the filtrate, add 25 ml of distilled water and 25 ml of 0.4 N lead (II) acetate, stir for 5 minutes, and filter. The filtrate was extracted three times

with a 20 mL combination of three parts chloroform and two parts isopropanol. The juice batch is evaporated at a maximum temperature of 50 degrees Celsius. The remaining is dissolved in 2 millilitres of methanol. The residual solution was used in the next experiments; precisely, 0.1 ml of the experimental solution was placed in a test tube and evaporated in a water bath. The residue is dissolved in 2 mL of distilled water and 5 drops of Molisch reagent before gradually adding 2 mL of concentrated sulphuric acid. Glycosides in plants are regarded good if a purple ring forms.

2.4.4. Saponin Test

A test tube contained 0.5 grams of dry powder and 10 millilitres of hot water, which was then filtered. The solution or filtrate is transferred to a test tube and vigorously stirred for 10 seconds. If a stable foam forms in the test tube for at least 10 minutes, reaching a height of 1-10 cm and persisting when numerous drops of 2 N hydrochloric acid are added, saponins are present.

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2.4.5. Tannin Test

Extract 0.5 grams of dry powder with 10 millilitres of distilled water, then dissolve and dilute the filtrate with distilled water until colourless. Measure out 2 ml of the solution and add 1-2 drops of the 3% iron (III) chloride reagent. The presence of blue or black pigmentation suggests the presence of tannins.

2.4.6. Steroid/Triterpenoid Test

A gram of dry powder was carefully weighed, macerated with 20 ml of n-hexane for two hours, and then filtered. The filtrate evaporates in an evaporating cup. Add 2 drops of acetic anhydride and 1 drop of concentrated sulphuric acid to the remainder. Steroids appear blue or green, but triterpenoids appear red, pink, or purple.

2.5. Preparation of Soka Flower Ethanol Extract

Four hundred grams of dried powder were placed in a vessel and combined with 3000 ml of 96% ethanol. The vessel was sealed and left for five days in a light-protected environment, with occasional stirring. The pulp underwent maceration with 25 parts (1,000 ml) of 96% ethanol in a sealed vessel, maintained in a cool, light-protected environment for 2 days, with periodic stirring and dusting. The combined filtrate is concentrated using a rotary evaporator at a temperature range of 40-50°C until a viscous extract is achieved.

2.6. Standarization of Soka Flower Ethanol Extract

The standardization of the ethanol extract involves assessing the moisture content, quantifying the total ash concentration, and evaluating the acid-insoluble ash content. The extract standardization technique adheres to the dry characterization protocol.

2.7. Phytochemical Screening of Soka Ethanol Extract

The phytochemical screening procedure for the ethanol extract of soka flower was in accordance with the phytochemical screening procedure for soka dry sample powder.

2.8. Tools Sterilization

The tools to be sterilized must first be washed and dried. The petri dish is wrapped in parchment paper for glassware (test tube, beakerglass, Erlenmeyer) covered with sterile cottoncovered with sterile gauze, then wrapped in parchment paper, sterilized in the oven at 150°C for 2 hours. Gauze, cotton, string, measuring cup, pipette and object glass were also wrapped in parchment paper and sterilized by autoclaving at121°C with 1atm pressure for 15 minutes. For tools such as loops, L rods, tools made of rubber and sterilized tweezers soak in 70% alcohol for 5 minutes then ignite with a Bunsen flame.

2.9. Fungal Culture

2.9.1 Propagation of Fungal Cultures of Candida albicans and Pityrosporum ovale

Stock cultures of *Candida albicans* and *Pityrosporum ovale* fungi were made by taking a loop of fungal colonies using a sterile loop needle, then planting them in oblique Sabouraud Dextrose Agar (SDA) media by scraping, then incubating them in an incubator at 25°C for 48 hours [8].

2.9.2 Preparation of Medium and Fungal

Five milliliters of Sabouraud Dextrose Agar solution were dispensed into each sterile test tube and thereafter sealed with aluminum foil. The medium was sterilized in an autoclave at 121°C for 15 minutes, then allowed to cool at ambient temperature for approximately 30 minutes until it solidified at 30°C. Fungal preparation involves obtaining a loop of a mushroom colony with a sterile loop needle, streaking it onto an oblique Sabouraud Dextrose Agar (SDA) medium, and incubating it at 25°C for 48 hours [8].

2.9.3 Production of Fungal Inoculums

Stock cultures of *Candida albicans* and *Pityrosporum ovale* were obtained with a sterile loop needle and subsequently suspended in a test tube with 10 ml of Sabouraud Dextrose broth (SDB). The transmittance was then measured at 530 nm using a UV-Visible spectrophotometer. The transmittance value is roughly 90%, which will be utilized to standardize the volume of stock suspension to be incorporated into the inocula agar layer.

2.9.4 Preparation of Test Solutions With Various Concentrations

Five grams of ethanol extract from soka flower were dissolved in DMSO and then diluted to a final amount of 10 ml, yielding a concentration of 500 mg/ml after vigorous stirring. Subsequent dilutions produced ethanol extracts with concentrations of 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 8.5, 7.5, 6.5, and 5.5 mg/ml.

2.9.5 Antifungal Activity

One milliliter of inoculum was added to a petri dish, followed by 15 milliliters of frozen sterile SDA media. The petri dish was homogenized on the table surface of the laminar airflow cabinet to ensure that the media and mushroom suspension were evenly mixed prior to solidification. Paper scraps drenched with varied amounts of a test solution made from the ethanol extract of soka flowers were placed on top of the compacted material. Dimethyl sulfoxide (DMSO) served as a negative control, whereas 2% ketoconazole was used as a positive control. The assembly was placed into a Petri dish and sealed. Allow it to rest for 10-15 minutes. Then it was incubated at 25°C for 48 hours. The antifungal activity of the ethanol extract from soka flowers was assessed by measuring the size of the clean area surrounding the reservoir with a vernier caliper. This experiment was repeated three times [8].

2.10. Statistical Analysis

The data on the diameter of the inhibitory zone on fungi growth were reported as mean ± standard deviation, followed by tests for normality and homogeneity of distribution. If the data follows a normal distribution, it is evaluated using a one-way ANOVA test. If the data is not normally distributed, it is subjected to the Kruskal-Wallis test. The analysis utilized SPSS software version 22 at a 95% confidence level.

3. Result and Discussion

3.1. Macroscopic of Soka Flower

Macroscopic observation aims to see the character of the plant part itself by watching the identifier fragment, which is a specific component of the plant. The results of macroscopic examination were carried out on fresh soft-shelled flowers, dry soft-shelled flowers, powdered soft-shelled flowers, and soka flower dry powder. In the soka flower, the shape of the flower stem is like a tube with a length of 3-4 cm, has 4 petals with an oval to ovoid shape, is red in color, is odorless, and tastes slightly bitter. In Soka flower dry powder, the flower stem looks like a slightly curved tube with a length of 3-4 cm and has 4 flower petals with an oval to ovoid shape, brownish red in color, odorless, and tasteless. Meanwhile, the dry powder is red-brown in color, has a characteristic odor, and is tasteless. The results of the inspection can be seen in Figure 1 below.

Figure 1. (a) Macroscopic Examination of Fresh Soka Flowers; (b) Soka Flower Dry Macroscopic Examination; (c) Soka Flower Dry Powder Macroscopic Examination.

The microscopic test is designed to examine the identifying fragments of the dry dryplant under a microscope. The results of microscopic inspection of the simplex soka flower. Flower collected epidermal tissue, parenchymal tissue, parasitic stomata, and unicellular trichomes. The image below shows the findings of the inspection.

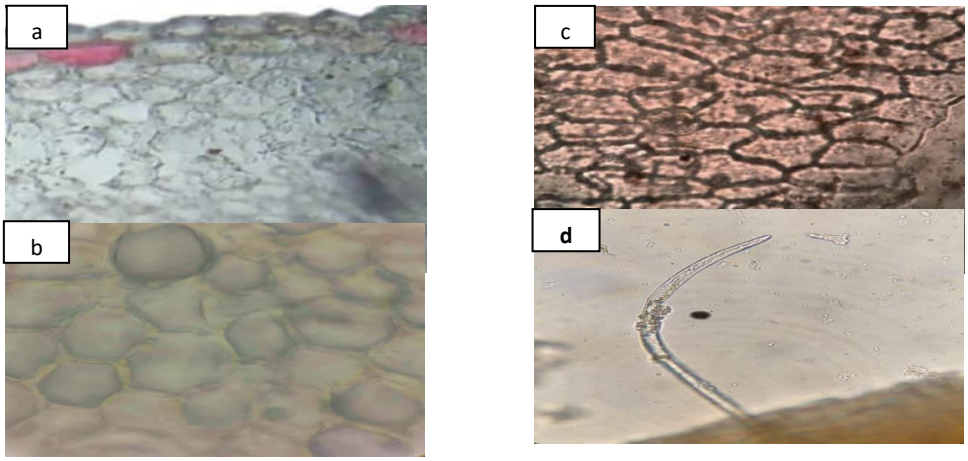

Figure 2. Microscopy of soka flower dry powder 10x10 (a)Epidermis; (b) Parenchyma Tissue; (c) Parasitic stomata; (d) Unicellular trichomes.

3.2. Result of Characterization of Soka Flower Dry Powder

The desiccated soka blossom's characterisation study yielded data on water content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content. The dry characteristics study aims to evaluate the powdered test material's quality and safety.

Table 1. Characterization of soka flower dry powder.

No.	Parameter	Amount
1	Water content	3,99% ± 0,00
2	Water soluble extract content	24,30%±0,23
3	Ethanol soluble extract content	20,11%±0,16
4	Total ash content	3,72%±0,03
5	Acid insoluble ash content	0,69%±0,01

Table 1 shows that the dry powder had a moisture content of 3.99%. The results met the dry moisture content standards, which are not to exceed 10%. The analysis found that the dry sample contained 24.30% water-soluble extract and 20.11% ethanol-soluble extract. The assessment of water-soluble and ethanol-soluble extract content is intended to quantify the extract yield in each solvent. Carbohydrates, salts, certain vitamins, and certain chemical compounds are all water-soluble. The identification of the item to be extracted is critical because it provides insight into the amount of dissolved material with therapeutic properties.

The total ash content of the dried simplex flower was 3.72%, whereas the acid-insoluble ash content was 0.69%. The ash content present in a plant does not consist entirely of chemicals detrimental to human health. Calcium, zinc, iron, salt, and potassium are metallic minerals that offer numerous advantages for human health. According to Zahra et al. (2015), Assess the concentrations of inorganic substances that are insoluble in acid, including silica, lead, and mercury metals. You can read the acid-soluble ash concentration as the presence of silicate contaminants, such as soil and sand [10].

3.3. Phytochemical Screening of Soka Flower Dry Powder

Phytochemical screening is a method used to check for chemical compounds or secondary metabolites in plants by putting the sample in a test tube and then adding a detection reagent.

Table 2. **P**hytochemical screening of soka flowerdry powder.

No.	Parameter	Amount
1.	Alkaloids	-
2.	Tannins	+
3.	Saponins	+

4.	Flavonoids	+
5.	Steroids/Triterpenoids	+
6.	Glycosides	+

The results indicated that the simplex flower dry positively contains tannins, saponins, flavonoids, steroids/triterpenoids, and glycosides.

3.4. Extraction

The processing of 3.08 kg of fresh soka flowers yielded 535 grams of dried product, resulting in a yield of 17.37%. We used 4 L of 96% ethanol to macerate up to 400 g of material. The maceration results were evaporated with a rotary evaporator at 50°C, yielding a viscous extract of 73 g (18.25% yield). The yield values obtained align with the research undertaken by Maniyar et al. (2010), which is 18.6%. Yield assesses the ratio of the weight of the generated extract to the quantity of dry powder extracted. We assess the yield to quantify the amount of extracted active chemicals. A higher yield results in an increased extraction of active chemicals [11].

3.5. Extract Characterization

The objective of extract characterisation is to guarantee that the finished product maintains consistent parameter values to assure the safety, quality, and efficacy of raw materials. The results are presented in Table 3 below.

Table 3. Characterization of soka flower ethanol extract

No.	Parameter	Amount
1.	Water content	7,95% ± 0,00
2.	Total ash content	2,65 % ± 0,03
3.	acid insolubleash content	0,45 % ± 0,01

The analysis of the water content in the ethanol extract of the soka flower yielded a result of 7.95%. Thick extracts possess a water content ranging from 5% to 30%. Inserting the sample into a test tube and subsequently introducing the detecting reagent. Excessive water content promotes microbial growth, hence diminishing the stability of the extract. The overall ash concentration from the ethanol extract of soka flower was 2.65%, whereas the acid-insoluble ash content was 0.45%.

3.6. Phytochemical Screening of Soka Flower Ethanol Extract

The objective of phytochemical screening is to identify the presence of secondary metabolites within a given sample. The findings from the phytochemical screening are presented in table 4 below.

Table 4. Phytochemical screening of soka flower ethanol extract.

No.	Parameter	Result
1.	Alkaloids	-
2.	Tannins	+
3.	Saponins	+
4.	Flavonoids	+
5.	Steroids/Triterpenoids	+
6.	Glycosides	+

Table 4 shows that the ethanol extract of soka flower is high in tannins, saponins, flavonoids, steroids/triterpenoids, and glycosides. The phytochemical tests revealed that flavonoids were found because the amyl alcohol layer turned orange, saponins by a 3 cm foam height, glycosides by a purple ring in the glycone test, tannins by a green color, and steroids/triterpenoids by a purple color.

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3.7. Antifungal Activity of Soka Flowers Ethanol Extract

The findings regarding the antifungal activity of soka flower ethanol extract are presented in Table 5 below.

Table 5. Antifungal activity of soka flower ethanol extract against *Candida albicans* and *Pityrosporum ovale.*

Concentration(mg/ml)	*Candida albicans*	*Pityrosporum ovale*
	Inhibitor Zone (mm)	Inhibitor Zone (mm)
400	18,1±0,25	14,7±0,55
300	16,1±0,45	13,8±0,17
200	15,7±0,45	13,4±0,35
100	14,7±0,32	12±0,30
90	13,8±0,70	11,7±0,5
80	12±0,52	11,2±0,10
70	11,4±0,15	11,1±0,55
60	10,9±0,17	10,9±0,50
50	10,4±0,25	10,7±0,50
40	10,4±0,25	-
30	10,2±0,26	-
20	10,0±0,35	-
10	9,9±0,30	-
8,5	9,5±0,28	-
7,5	9,4±0,32	-
6,5	9,1±0,32	-
5,5	8,8±0,05	-
Positive control (Ketonazole 2%)	19,4±0,30	14,8±0,40
Negative control (DMSO)	-	-

The antifungal activity showed that the ethanol extract from soka flowers against *Candida albicans and Pityrosporum ovale* revealed which the minimum inhibitory concentration (MIC) for *Candida albicans* was 5.5 mg/ml, with an inhibition zone diameter of 8.8 mm. In comparison, *Pityrosporum ovale* has MIC was 50 mg/ml, with an inhibitory zone diameter of 10.7 mm. The negative control, DMSO, displayed no inhibition zone, whereas the positive control, 2% ketoconazole, produced an inhibition zone diameter of 19.4 mm against *Candida albicans* and 14.8 mm against the *Pityrosporum ovale*. The diameter of the inhibition zone was measured and shown to be directly proportional to the concentration of the extract; especially, as the concentration of the extract increased, so did the size of the inhibition zone.

The Indonesian Pharmacopeia Edition IV indicates that a satisfactory inhibition zone measures around 14 mm to 16 mm in diameter, demonstrating a reproducible dose relationship. The effective concentration of the ethanol extract of the soka flower on *Candida albicans* is 100 mg/ml, resulting in a 14.7 mm broad inhibitory zone. In comparison, *Pityrosporum ovale* has an effective concentration of 400 mg/ml. The inhibitory zone's diameter is recorded as 14.7 mm. Davis and Stout (1971) divided the antimicrobial inhibition zone into four categories: no activity (<5 mm), moderate activity (5-10 mm), strong activity (10-20 mm), and high activity (>20 mm). *Candida albicans* is classified as having significant antifungal activity at a dosage of 20 mg/ml, resulting in an inhibition zone diameter of 10.0 mm. At a dosage of 50 mg/ml, *Pityrosporum ovale* exhibits high antifungal activity, resulting in an inhibition zone diameter of 10.7 mm. The formation of inhibition zones in the two mushrooms is thought to come from the plant chemicals or secondary substances found in the ethanol extract of soka flowers.

The phytochemical screening results showed that the ethanol extract of soka (*Ixora chinensis* Lam.) included a variety of chemical components, including tannins, saponins, flavonoids, steroids/triterpenoids, and glycosides. The antifungal activity of these secondary metabolites is achieved by a variety of methods. Tannins operate as antifungals by blocking extracellular enzymes such as cellulase, pectinase, and lactase. They also cause a lack of nutritional substrates, such as metal complexes and insoluble proteins, which reduces fungal membrane activity and inhibits oxidative phosphorylation [12].

Saponins have antifungal efficacy that is comparable to or even greater than that of traditional antifungal drugs, and some saponins are effective against drug-resistant strains. Saponins have considerable antifungal action against *Candida albicans* and demonstrate synergistic antifungal effects. Saponins are a promising alternative antifungal drug due to their high effectiveness and safety [13].

The extract contains chemicals with antibfungal activity. We refer to these compounds as flavonoids. Flavonoids' biological effects are mediated by a variety of metabolic pathways and interactions. Antimicrobials can be divided into three categories based on how they inhibit nucleic acid production. Inhibition of cell membrane function has an impact on energy metabolism [14]. Steroids are fat-loving compounds that can serve as antifungals by inhibiting the formation of ergosterol.

The results showed that *Candida albicans* has a larger inhibitory zone than *Pityrosporum ovale*. Stalhberger et al. (2015) report that *Pityrosporum ovale* has a thick and inert multilamellar cell wall. The multilamellar layer, which includes the capsule, contains 15-20% fat [17], whereas the fungus *Candida albicans* has 1-7% fat (Drasar, 2003). As a result, high amounts of extract are necessary for its destruction.

At the base of the cell wall, a configuration of serrations invaginates into the cell membrane. *Pityrosporum ovale* possesses a cell wall with a helicoid pattern, showcasing a complex and dynamic structure that efficiently protects cells from osmotic pressure variations and environmental stress. Conversely, the cell wall architecture of Candida albicans exhibits fibrils organized in both parallel and perpendicular orientations [19]. *Pityrosporum ovale* can generate biofilms consisting of an extracellular matrix formed by polysaccharides, amyloid, and fibers [20]. This matrix is essential for protecting the fungus against antifungal drugs via multiple methods. Persister cells, related to spore-like cells, initially grow and synthesize proteins that safeguard antifungal targets. Moreover, modifying growth and metabolism, as well as mutation frequency and gene transfer. Third, the extracellular matrix will function as a diffusion barrier for tiny compounds [21].

The statistical study, which was carried out using SPSS, sought to determine whether there was a significant difference in extract concentration vs inhibitory zone diameter. If the data has a normal distribution, we will do a one-way ANOVA test. When data deviates from a normal distribution, we use the Kruskal-Wallis test for analysis. The normality test findings for the concentration of ethanol extract of soka flowers and the size of inhibitory zones for *Candida albicans* and *Pityrosporum ovale* were less than 0.05. This result implies that the data does not follow a normal distribution, as demonstrated by the Shapiro-Wilk test. When the data does not follow a normal distribution, use the Kruskal-Wallis test. The results of 49 Kruskal-Wallis tests demonstrate a significant difference in the quantities of ethanol extract from soka flowers based on the size of the inhibitory zones for *Candida albicans* and *Pityrosporum ovale*, with probabilities of 0.000 and 0.001, respectively, both less than 0.05.

4. Conclusion

The study discovered that *Candida albicans* is 5.5 mg/ml with an inhibition zone diameter of 8.8 mm ±0.05, while for *Pityrosporum ovale*, the minimum inhibitory concentration is 50 mg/ml with an inhibition zone diameter of 10.7 mm ±0.50. The statistical analysis from Kruskal-Wallis tests show a significant difference in the amounts of ethanol extract from soka flowers based on the size of the inhibition zones for *Candida albicans* and *Pityrosporum ovale*, with probabilities of 0.000 and 0.001, both of which are below 0.05.

5. Acknowledgments

This research is funded by private individuals

6. Conflict of Interest

All authors declare that there are no conflicts of interest.

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