

DOI: <https://dx.doi.org/10.18203/2319-2003.ijbcp20233892>

Original Research Article

## Antifungal activity testing of curry leaf ethanol extract on the growth of *Pityrosporum ovale* and *Candida albicans* fungus

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**Received:** 01 December 2023

**Revised:** 18 December 2023

**Accepted:** 20 December 2023

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### ABSTRACT

**Background:** The curry leaf is an Indonesian plant commonly utilized as a spice. Curry leaves are abundant in secondary metabolites, which endow this plant with numerous advantages, including antibacterial and antifungal properties, as well as the ability to reduce blood sugar levels and blood pressure. The objective of this study is to assess the efficacy of an ethanol extract derived from curry leaves in suppressing the proliferation of *Pityrosporum ovale* and *Candida albicans* fungus.

**Methods:** The symbiotic properties and phytochemical composition of curry leaf simplisia were examined. The antifungal efficacy of the ethanol extract derived from curry leaves was evaluated against *Pityrosporum ovale* and *Candida albicans* using the disc diffusion method. Calculate the precise values of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).

**Results:** The simplisia of curry leaf fulfills the criteria for simplisia characterization. Curry leaves possess a variety of secondary metabolite chemicals, including alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids. The activity against the inhibition of fungal growth of *Pityrosporum ovale* and *Candida albicans* can suppress fungal growth with inhibition zone diameters measuring  $6.93 \pm 0.15$  mm and  $7.27 \pm 0.47$  mm, respectively. The minimum lethal concentration of leaf ethanol extract for *Pityrosporum ovale* fungi is 8.75%, resulting in a decrease of 98.25%. For *Candida albicans* fungi, the minimum lethal concentration is 12.5%, resulting in a reduction of 98.37%.

**Conclusions:** The ethanol extract derived from curry leaves has the ability to hinder the proliferation of *Pityrosporum ovale* and *Candida albicans* fungus.

**Keywords:** *Pityrosporum ovale* fungus, *Candida albicans* fungus, MIC, MBC, Curry leaf

### INTRODUCTION

Traditional medicine is an emerging discipline of study and practice that offers an alternative approach to treatment, focusing on research and education. The utilization of natural materials is a conventional kind of therapy. Traditional medicine is often regarded as a favorable alternative treatment for the community due to its minimal adverse reactions and its efficacy in delivering therapeutic results.<sup>1</sup>

Fungi and bacteria are pathogens responsible for several ailments. Causes of infection resulting from bacterial or fungal pathogens fungi are a significant contributor to infectious diseases, particularly in tropical regions where the moist climate promotes the proliferation of fungal organisms.<sup>2</sup> Indonesia is a tropical nation characterized by high temperatures and humidity, which create favorable conditions for the proliferation of fungi, including both non-pathogenic and pathogenic species. The rise in patients with fungal infections can be attributed to several

predispositions, such as the general disregard for personal hygiene and the widespread use of antibiotics.<sup>3</sup>

Two pathogenic fungi are *Pityrosporum ovale* and *Candida albicans*. *Candida albicans* is the predominant fungal infection in humans, particularly in the oral cavity. *Candida albicans* is a pathogenic fungus belonging to the Deuteromycota group. *Candida* species are responsible for causing candidiasis, an opportunistic infection that affects the skin, mucosa, and internal organs of humans. This species exhibits ovoid or spherical shape with a diameter ranging from 3 to 5 µm and has the ability to generate pseudo-hyphae. *Candida albicans* species exhibit two distinct morphologies: a yeast-like form and a hyphal-like form.<sup>4</sup> *Pityrosporum ovale* is a unicellular yeast or fungus classified as a member of the *Malassezia* sp genus and the *Cryptococcaceae* family. *Pityrosporum ovale*, a microorganism, is believed to be the primary culprit behind dandruff. This fungus is often present on the scalp as part of the regular flora. However, under hair conditions characterized by an overabundance of oil glands, this fungus can thrive and multiply rapidly.<sup>5,6</sup> The issue of infection is presently resolved through the utilization of antibiotics. Prolonged use of antibiotics can lead to antibiotic resistance, necessitating an initial irritation test for certain patients to ensure the safety of antibiotic formulations. Therefore, several alternatives are needed to overcome the infection naturally. A significant number of individuals currently experience the issue of diminished self-assurance due to dandruff problems. Dandruff is mostly caused by a fungal infection affecting the scalp.<sup>7</sup> Therefore, an alternative is needed to overcome the fungus that causes dandruff naturally.

Prior to the widespread utilization of modern medicine by the general populace, traditional medicine was widely recognized and implemented. The application and research of traditional medicine in Indonesia is presently experiencing a surge. Indonesia possesses several botanical species with therapeutic characteristics, although their scientific exploration remains limited. Conducting studies on plants utilized in traditional medicine is vital to validate their effectiveness and acquire scientific knowledge regarding the active constituents of plant materials.<sup>8</sup> An alternate approach in the field of medicine is the extraction and utilization of bioactive antifungal compounds derived from curry leaves.

Curry leaves, originating from India, are a prevalent plant in Indonesia and are extensively utilized as a flavoring. The antibacterial effects of curry leaf extract against both gram-positive and gram-negative bacteria are attributed to the presence of carbozole alkaloid chemicals.<sup>9</sup> Curry leaves not only yield extracts but also generate essential oils, which include antimicrobial properties that effectively hinder the proliferation of bacteria and fungus such as *Proteus mirabilis*, *Corynebacterium pseudotuberculosis*, and various other fungi. *Listeria innocua*, *Enterococcus faecalis* and *Salmonella typhimurium*.<sup>10-12</sup> The test findings demonstrate the

efficacy of curry leaves as both an antibacterial and antifungal agent.

Given this context, the objective of this study is to assess the efficacy of the ethanol extract derived from curry leaves in determining the MIC and the minimum fungicidal concentration (MFC) against the growth of *Pityrosporum ovale* and *Candida albicans* fungi.

## METHODS

The research period will be carried out from March to June 2023. Carried out at the microbiology and parasitology laboratory, faculty of pharmacy, universitas Sumatera Utara.

### Equipment and supplies

The research employs many instruments including glassware, an autoclave, an oven, a caliper, a drying cabinet, and a hot plate. The materials utilized in this study include curry leaves, 96% ethanol, distilled water, potato dextrose agar (PDA), plate count agar (PCA), peptone dilution fluid (PDF), Sabouraud dextrose agar (SDA), sabouraud dextrose broth (SDB), *Candida albicans* and *Pityrosporum ovale* test fungi, carbopol, glycerin, triethanolamine, methyl paraben, propyl paraben, dimethicone, and DMSO (dimethyl sulfoxide).

### Preparation of simplisia and ethanol extract of curry leaf (*Murraya koenigii* L.)

After being thoroughly cleaned, curry leaf samples are dried in a drying cabinet until they are completely dry. The weight of the curry leaf sample is measured as dry weight once it has dried. The simplisia made from curry leaves is subsequently crushed into smaller pieces and immersed in an ethanol solvent. Stir it periodically and let it sit for five days. The filtrate and residue were then separated by filtering. A rotary evaporator is used to concentrate the collected filtrate.<sup>10</sup>

### Characteristic testing and phytochemical screening of curry leaf simplisia (*Murraya koenigii* L.)

Curry leaves undergo characteristic testing to assess the quality of the simplisia intended for use. The conducted characteristic tests include analysis of water content, ethanol-soluble juice content, water-soluble juice content, total ash content, and acid-insoluble ash content. The curry leaves underwent phytochemical screening assays to detect the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids.<sup>13</sup>

### Antifungal MIC testing of *pityrosporum ovale* and *Candida albicans*

The antifungal activity test was conducted using the agar diffusion method using disc paper. A sterile petri dish was filled with 100 µL of *Candida albicans* fungal inoculum.

Then, 10 mL of thawed Sabouraud dextrose agar (SDA) media was added and the mixture was allowed to reach a temperature of 45 °C. The mixture was then homogenized and left to harden. Curry leaves were extracted with ethanol and the resulting extract was applied onto sterile paper discs. The discs were then placed on the media surface for a duration of 30 minutes. The disc paper was carefully positioned onto the media surface using tweezers and gently applied pressure. Subsequently, it was placed in an incubator set at a temperature of 37 degrees Celsius for a duration of 24 hours. Subsequently, the diameter of the inhibitory zone, also known as the clear zone, was quantified using a digital caliper and reported in millimeters. An identical experiment was performed using *Pityrosporum ovale* bacteria.<sup>14,15</sup>

#### Antifungal minimum kill concentration testing of *Pityrosporum ovale* and *Candida albicans*

The purpose of the test was to ascertain the least concentration of curry leaf ethanol extract, known as the minimum kill concentration (MMC), that is effective against *Candida albicans* and *Pityrosporum ovale*. Each test tube containing SDB media was supplemented with 1 mL of fungal inoculum and subsequently agitated using a vortex. Subsequently, a fungal suspension solution was supplemented with 1 mL of ethanol extract of curry leaves at different doses. The tubes were subjected to incubation at a temperature of 37 °C for a duration of 24 hours, after which the turbidity of the incubation solution was visually examined. The homogenized incubation solution was dispensed in 50 µL aliquots for each concentration. These aliquots were then added to solid medium of SDA and spread evenly using a swab. The media was thereafter incubated at 37°C for 24 hours. The minimum concentration at which no fungal colonies were observed was designated as KBM.<sup>3,14,16</sup>

#### Data analysis

The study's data were analyzed using SPSS software, employing the one-way ANOVA approach and the Duncan test as a post-hoc analysis. In order to obtain reliable analytic outcomes, researchers utilized the SPSS software to conduct this inferential study. The findings of this inferential analysis will be utilized by researchers to establish the statistical significance, or lack thereof, of the hypothesis they have formulated.

## RESULTS

#### Phytochemical screening test results and characterization of curry leaf simplisa

The findings of the analysis conducted on the curry leaf sample are presented in Table 1.

The results of the phytochemical screening of curry leaves can be seen in Table 2.

**Table 1: Characteristic data of curry leaf simplisia.**

Category	Result (% ± SD)
Water content	8.64±0.12
Water soluble essence content	36.06±0.96
Ethanol soluble juice content	18.72±1.12
Total ash content	6.67±0.67
Acid insoluble ash content	1.38±0.82

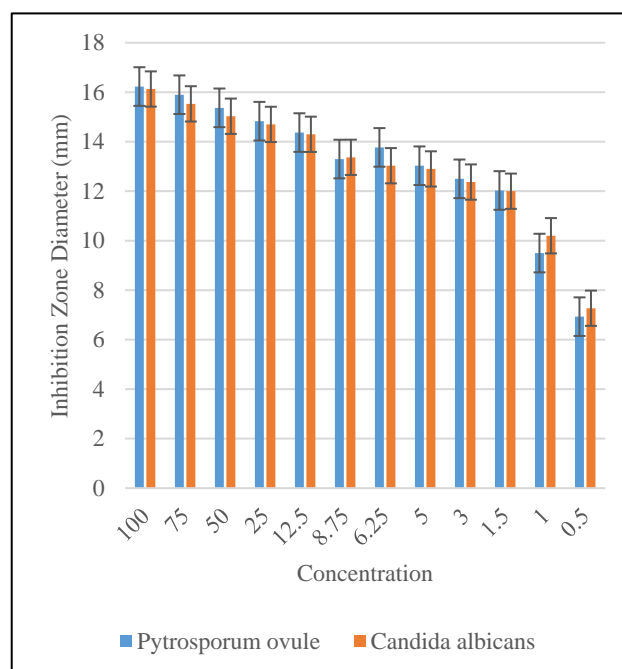
**Table 2: Phytochemical screening data of curry leaf simplisia.**

Category	Result (% ± SD)
Alkaloids	Positive
Flavonoids	Positive
Glycosides	Positive
Saponins	Positive
Tannins	Positive
Triterpenoids/steroids	Positive

#### Test results of ethanol extract of curry leaves against the growth of *Pityrosporum oval* and *Candida albicans* fungi

#### MIC of curry leaf ethanol extract for antifungal *Pityrosporum ovale* and *Candida albicans*

The ethanol extract of curry leaves exhibited antifungal efficacy against the growth of fungus *Pityrosporum ovale* and *Candida albicans*.



**Figure 1: Effect of curry leaf ethanol extract concentration on the growth inhibition of *Pityrosporum ovale* and *Candida albicans*.**

Table 3 below displays the information.

Minimum kill concentration of curry leaf ethanol extracts antifungal *Pityrosporum ovale* and *Candida albicans*

Data on the results of the minimum kill concentration of

the curry leaf (*Murraya koenigii*) ethanol extracts against the growth of *Pityrosporum ovule* fungus as well as the *Candida albicans* fungus can be described in the Table 4 below.

**Table 3: Measurement data of zone of inhibition diameter of *Pityrosporum oval* and *Candida albicans*.**

No	Concentration (%)	Inhibition zone diameter (mm) (n ± SD)	
		<i>Pytrosporum ovale</i>	<i>Candida albicans</i>
1.	100	16.23±0.32	16.13±0.31
2.	75	15.90±0.26	15.53±0.25
3.	50	15.37±0.31	15.03±0.35
4.	25	14.83±0.42	14.70±0.36
5.	12.5	14.37±0.47	14.30±0.36
6.	8.75	14.30±0.56	13.73±0.06
7.	6.25	13.77±0.65	13.03±0.38
8.	5	13.03±0.31	12.90±0.56
9.	3	12.50±0.21	12.37±0.36
10.	1.5	12.03±0.60	12.00±0.44
11.	1	9.50±0.72	10.20±0.20
12.	0.5	6.93±0.15	7.27±0.47

**Table 4: Data on the minimum concentration of curry leaf ethanol extract required to kill the fungus *Pityrosporum ovale* and *Candida albicans***

Sample (N <sup>o</sup> )	<i>Pytrosporum ovale</i>				<i>Candida albicans</i>			
	Count	Difference	% reduction	Log reduction	Count	Difference	% reduction	Log reduction
K	5322	0.00	0.00	0.00	4858	0.00	0.00	0.00
0.5	3241	2081	39.10	1.59	2336	2522	51.91	1.72
1	1302	4020	75.54	1.88	1990	2868	59.04	1.77
1.5	847	4475	84.04	1.92	1014	3844	79.13	1.90
3	400	4922	92.48	1.97	487	4317	89.98	1.95
5	262	5060	95.08	1.98	306	4552	93.70	1.97
6.25	124	5198	97.67	1.99	262	4596	94.61	1.98
8.75	93	5229	98.25	1.99	109	4749	97.76	1.99
12.5	60	5262	98.87	2.00	79	4779	98.37	1.99
25	48	5274	99.10	2.00	56	4802	98.85	1.99
50	31	5291	99.42	2.00	39	4819	99.20	2.00
75	24	5298	99.55	1.98	28	4830	99.42	2.00
100	7	5315	99.87	2.00	9	4849	99.81	2.00

## DISCUSSION

### *Phytochemical screening test results and characterization of curry leaf simplisia*

Characterization testing is conducted to verify the quality of the sample intended for use. The sample must be devoid of contaminants, and it should not contain any metallic or mineral residues that could disrupt the study procedure.

The analysis of water content is conducted to ascertain the lower threshold of moisture content for samples that have undergone the drying process. The allowable water-soluble juice concentration in dried simplisia is less than 10%. The composition of curry leaves was analyzed to determine the number of chemicals that can be dissolved in water and ethanol solvents. Based on the conducted

experiments, the metabolite chemicals found in curry leaf content exhibited higher solubility in water solvents, specifically 36.06%, as opposed to 18.72% in ethanol solvent.<sup>17</sup> The analysis of water content is conducted to ascertain the lower threshold of moisture content for samples that have undergone the drying process. The allowable water-soluble juice concentration in dried simplisia is less than 10%. The composition of curry leaves was analyzed to determine the number of chemicals that can be dissolved in water and ethanol solvents. Based on the conducted experiments, the metabolite chemicals found in curry leaf content exhibited higher solubility in water solvents, specifically 36.06%, as opposed to 18.72% in ethanol solvent.<sup>18</sup>

The findings of this study are consistent with earlier studies demonstrating the presence of the same secondary

metabolites in curry leaves as the subject of this investigation. The state of the fertilizer, the age of the sample plants utilized, the location of the plants, and the state of the soil where they are grown are some of the variables that can affect the results of secondary metabolite screening. The amount of secondary metabolites in a single plant might vary depending on a few of these parameters.<sup>19</sup>

The analysis of the test findings revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoid/steroid chemicals in the ethanol extract of curry leaves. Phytochemical analysis is a method used to ascertain the presence and quantity of secondary metabolites in a given sample.<sup>20</sup> Secondary metabolite chemicals, particularly flavonoids, possess the ability to eradicate and dismantle bacterial cell walls, hence impeding the growth of fungi or bacteria. Saponin chemicals function as exfoliants, removing dead skin cells, which in turn unclogs scalp pores and inhibits the development of dandruff on the scalp.<sup>21</sup> The ethanol extract of curry leaves possesses antifungal properties and contains senyawa alkaloids, flavonoids, saponins, and tannins.<sup>8</sup> The inhibitory mechanism in the extract is dependent on the active chemicals present. Certain compounds inhibit by altering the process of nucleic acid production in cells, while others attack and inhibit fungal mitosis. This stimulation of hair roots leads to accelerated hair development.

#### **Test results of ethanol extract of curry leaves against the growth of *Pityrosporum oval* and *Candida albicans* fungi**

##### *MIC of curry leaf ethanol extract for antifungal Pityrosporum ovale and Candida albicans*

A number of earlier researchers have tested antifungals. Curry leaf ethanol extract was found to be a potent bacterial and fungal growth inhibitor in earlier studies. Previous studies have demonstrated that the ethanol extract of curry leaves has the ability to prevent *Candida albicans* fungus from growing inside the 18.23 mm inhibitory zone. The findings indicate that this research has not made much of an impact.<sup>8</sup> According to the findings presented in Table 3, curry leaf extract demonstrates the ability to impede the proliferation of *Pityrosporum ovalis* fungus, even at a concentration as low as 0.5%. At such dose, curry leaf extract exhibits inhibitory effects on the growth of *Pityrosporum ovale* fungus, but with a rather modest potency, as indicated by an inhibition zone diameter of 6.93 mm. Curry leaf extract, at doses of 1.5%, 3%, and 5%, exhibited inhibition zone widths of 12.03 mm, 12.50 mm, and 13.03 mm, respectively. This indicates that the extract has a strong inhibitory effect on *Pityrosporum ovale* fungi, demonstrating great sensitivity.<sup>22,23</sup> At a dosage of 0.5%, curry leaf extract effectively inhibits the growth of *Candida albicans* fungus, resulting in a weak inhibition zone with a diameter of 7.27 mm. Curry leaf extract, at doses of 1.5%, 3%, and 5%, shown suppression of *Candida albicans* growth. The

inhibition zone diameters were measured at 12.00 mm, 12.37 mm, and 12.90 mm, respectively. This indicates that the extract has a high sensitivity in inhibiting the development of *Candida albicans* fungi.

Previous research has demonstrated that curry leaf extract possesses the ability to hinder the growth of *Candida albicans* fungi. This inhibitory effect is observed at a minimal concentration of 6.25%, as the extract effectively suppresses fungal growth. No *Candida albicans* fungal growth was detected in samples containing curry leaf extract doses of 12.5%, 9.375%, and 8.75%, each tested with 4 repetitions. At a dosage of 8.75%, curry leaf extract exhibits complete inhibition of *Candida albicans* fungus. According to Muhammad et al the ethanol extract of curry leaf at concentrations of 12.5% and 25% has been identified as the most effective concentration for inhibiting the growth of *Candida albicans* fungus.<sup>8</sup>

Figure 1 demonstrates a positive correlation between the concentration of the ethanol extract of curry leaves and its antifungal activity. As the concentration increases, the diameter of the inhibitory zone generated likewise increases. The diameter of the inhibition zone is directly influenced by the concentration of the extract. Specifically, a higher concentration results in a larger inhibition zone diameter. Therefore, even the lowest concentration still exhibits antifungal action. The antifungal activity is classified as having low sensitivity if the diameter measures 6-9 mm, medium sensitivity if the diameter falls between 9-12 mm, and high sensitivity if the inhibition zone exceeds 12 mm.<sup>1,24,25</sup>

##### *Minimum kill concentration of curry leaf ethanol extract antifungal Pityrosporum ovale and Candida albicans*

Table 4 demonstrates that even when using a 100% ethanol extract of curry leaves, there are still fungal colonies present. The number of colonies seen is 7 and 9, resulting in a reduction of 99.87% and 99.81%, respectively. The ethanol extract of leaves exhibits a minimum lethal concentration of 8.75% against *Pityrosporum ovale* fungi, resulting in a decrease of 98.25%. Similarly, it demonstrates a minimum lethal concentration of 12.5% against *Candida albicans* fungi, with a reduction of 98.37%. The KBM value is derived by assessing the minimum concentration capable of reducing the number of colonies by 98-99% compared to the starting colonies (negative control).<sup>7</sup> The growth of *Candida albicans* fungi can be suppressed by curry leaf extract at concentrations of 12.5%, 9.375%, and 8.75%, as seen by the lack of fungal growth zones caused by *Candida albicans*.<sup>9,22</sup> Conversely, when the concentration reached 6.25%, an inhibitory zone was seen, with an average count of  $2.15 \times 10^4$  colony-forming units (CFU) per milliliter of *Candida albicans* fungus. There is an absence of inhibitory zones at concentrations of 5% and 3% due to the excessive number of *Candida albicans* fungal colonies, making it unfeasible to quantify. The efficacy of an antibiotic agent is influenced by various parameters, such as its

concentration. As the concentration increases, the antimicrobial efficacy also increases, as larger concentrations facilitate the dispersion of chemicals that effectively inhibit or kill germs.<sup>26</sup> According to this, the fungicidal activity of curry leaf ethanol extract can be enhanced from fungistatic to fungicidal as the concentration employed is increased. An effective antibiotic is characterized by its ability to inhibit germs while being present in a low-concentration state.<sup>27</sup>

## CONCLUSION

The research findings indicate that the ethanol extract derived from curry leaves had the ability to hinder the growth of *Pityrosporum ovale* and *Candida albicans* fungi.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee*

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**Cite this article as:** Sari UP, Reveny J, Sinaga SM. Antifungal activity testing of curry leaf ethanol extract on the growth of *Pityrosporum ovale* and *Candida albicans* fungus. Int J Basic Clin Pharmacol 2024;13:57-63.