



## Tlc Profile And Antifungal Activity Test Of Ethylacetat Extract Of The Flower Moon Herbs (*Tithonia diversifolia* (Hemsl.) Gray) Againts *Candida Albicans* And *Microsporum Canis*

Hepni<sup>1</sup>, Fitri Yanti<sup>2</sup>, Faisal Yusuf<sup>3</sup>

<sup>123</sup>Program Studi Farmasi, Sekolah Tinggi Ilmu Kesehatan (STIKes) Arjuna Laguboti, Jl. Y.P. Arjuna, Pintu Bosi, Toba, Sumatera Utara 22381, Indonesia

ARTICLE INFO	ABSTRACT
<p><b>Article history:</b></p> <p>Received March 11, 2022 Revised April 20, 2022 Accepted May 30, 2022</p>	<p>The moon flower herb is a plant species known and used as traditional medicine in Indonesia for generations. In previous studies it was known that moonflower (<i>Tithonia diversifolia</i> (Hemsl) Gray) has antihyperglycemic, antidiabetic, antimicrobial and antimalarial effects, and it is known that the roots, stems, leaves and essential oil of moonflower (<i>Tithonia diversifolia</i> (Hemsl) Gray) contain alkaloids, compounds phenolics, steroids, saponins and flavonoids. The purpose of this study was to determine the activity of ethylacetate extract of moonflower herb against <i>Candida albicans</i> and <i>Microsporum canis</i>. This research was carried out in several stages, starting with the process of collecting and processing samples, testing the characteristics of simplicia, maceration of the ethylacetate extract of the moonflower herb (<i>Tithonia diversifolia</i> (Hemsl) Grey), testing Thin Layer Chromatography (TLC) and testing antifungal activity. The results of the research that the moon flower herb has a water content of about 10.2%. Thin Layer Chromatography (TLC) test of moonflower herbs on ethyl acetate extract containing polyphenolic compounds, steroids and alkaloids. The minimum inhibitory concentration (MIC) of ethylacetate extract of moonflower herb against <i>Candida albicans</i> ATCC 10231 was at a concentration of 200 mg/mL, ie 3.00 mm, while in <i>Microsporum canis</i> it was found at a concentration of 300 mg/mL. which is equal to 13.21mm.</p>
<p><b>Keywords:</b></p> <p>Antifungal activity <i>Candida albicans</i> <i>Microsporum canis</i> Moonflower herb ethylacetate extract Thin layer chromatography</p>	

This is an open access article under the [CC BY-NC](https://creativecommons.org/licenses/by-nc/4.0/) license.



### Corresponding Author:

Hepni,  
Program Studi Farmasi,  
Sekolah Tinggi Ilmu Kesehatan (STIKes) Arjuna Laguboti,  
Jl. Y.P. Arjuna, Pintu Bosi, Toba, Sumatera Utara 22381, Indonesia  
Email: [hepni.bagariang89@gmail.com](mailto:hepni.bagariang89@gmail.com)

### 1. Introduction

Infectious diseases and antimicrobial drug resistance are problems that require great attention, therefore, it is necessary to conduct new research which is expected to be a solution to these problems. Sources of antimicrobials can come from plants that have the potential as antimicrobials in Indonesia, people have traditionally used a lot of plants to treat

various kinds of infectious diseases, but the use of traditional medicinal plants is still not widely supported by scientific research data (Soedarto, 2015).

*Microsporum canis* is one of the genera that causes dermatophytosis or tinea that infects the scalp the most (*Tinea Capitis*). Like other dermatophytes, *Microsporum canis* is able to break down keratin so that it can live on the skin in a non-invasive state such as keratinase, proteinase enzymes and fungal elastase are virulence factors (Indarjulianto et al., 2014). *Microsporum canis* is one of the most common causes of dermatophytosis or tinea that infects the scalp (*Tinea capitis*). Like other dermatophytes, *Microsporum canis* is able to break down keratin so that it can live on the skin in a non-invasive state such as keratinase, proteinase enzymes and fungal elastase are virulence factors (Fajri et al., 2018).

Candidiasis is a fungal infection caused by one of the *Candida* species, the most common being *Candida albicans*, which is commonly found in the oral cavity, digestive tract, reproductive tract and skin without causing disease or symptoms (Khatimah et al., 2018). In the oral cavity the number of *Candida albicans* ranges from 100-500 colonies per millimeter of saliva. The fungus *Candida albicans* will turn pathogenic when there are excessive amounts in the body. When the immune condition of the human body decreases, the fungus *Candida albicans* will cause candidiasis (Alfiah et al., 2015). Candidiasis (candidosis) is a group of infection caused by *Candida albicans* or other members of the *Candida* genus. These organisms usually infect skin, nails, mucous membranes, gastrointestinal tract, and can also cause systemic disease (Widasmaria et al., 2014)

According to (Sasmita et al., 2017) the ethanolic extract of *Tithonia diversifolia* has an anti-hyperglycemic effect and improves the effect of. According to (Sri Hartati Wahyuningsih et al., 2013), based on research conducted, the chloroform extract of the leaves of the flower moon has a cytotoxic effect on hela cells with an IC<sub>50</sub> value of 3.078 g/mL and a selectivity index of 26.09. Extracts of this plant are also known to have antimalarial and antimicrobial effects. A previous study by (Utami, W.S., 2012), reported the antimalarial effect of *Tithonia diversifolia* leaf methanol extract against *Plasmodium falciparum* mosquito larvae.

Moonflower is one type of *simplicia* known in Indonesia. The people of Sumatra usually use this plant as a fever medicine and wound medicine, but they still consider this plant as a weed in their planting area. Moonflowers are plants such as sunflowers with a height of no more than one meter. The green leaves are curved and serrated sometimes like having three segments, the flowers are yellow like sunflowers but smaller and usually grow on the ground (Ningsih et al., 2016). Moonflower plants belonging to the *Compositae* or *Asteraceae* family can inhibit the growth of *Candida albicans*. In addition, this plant also has antimicrobials against gram-negative (G-) such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus stearothermophilus* and gram-positive (G+) bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Shigella* (Obafemi et al., 2006).

According to (Damayanti & Ervilita, 2019), in their research, after a phytochemical screening test was carried out, flower moon leaf extract reported that n-hexane extract had alkaloid and steroid compounds, ethylacetate extract had flavonoid and steroid compounds,

and ethanol extract had alkaloid, flavonoid, phenolic, and phenolic compounds. saponins and steroids.

Based on this, the researchers are interested in conducting research on "TLC profile and antifungal activity test of the n-hexane extract of the flower moon herb (*Tithonia diversifolia* (Hemsl.) Gray) against *Candida albicans* and *Microsporium canis*" to find out what compounds are contained in the extract. n-hexane from cauliflower herb in inhibiting the growth of *Candida albicans* and *Microsporium canis*. And to determine the optimal concentration of the n-hexane extract of the flower moon herb in inhibiting the growth of *Candida albicans* and *Microsporium canis*.

## 2. Research Methods

### 2.1 Nature of Research

The method used is the experimental method. The research phase includes the preparation of tools and chemicals to be used, identification of plants, preparation of plant samples, water content testing of *simplicia*, manufacture of extracts from the powder of the moonflower herb (*Tithonia diversifolia* (Hemsl.) Gray) by staged maceration using n-hexane solvent, ethylacetate, and ethanol, making reagent solutions. The class of chemical compounds was tested using the Thin Layer Chromatography (TLC) method and then tested for its potential as an antibacterial using the agar well method with a diameter of 8 mm using a metal shield against the fungi *Candida albicans* ATCC 10231 and *Microsporium canis*. The diameter of the inhibition zone was measured using a caliper. The parameter seen is the diameter of the growth inhibition seen in the clear area around the hole.

### 2.2 Tools and Materials Used

#### Tools used

The tools used in this research are glassware (Pyrex®), blender (Sijempol®), gas stove (Rinnai®), rough balance (Sun®), electric balance (Vibra Aj®), drying cabinet, autoclave (Fisons®), vortex mixer (K®), metal backer, caliper, filter paper, parchment paper, aluminum foil, knife, scissors, wire loop, Oven (Mettler®), water bath and rotary evaporator (Haake D®), aseptic cabinet, porcelain cup, desiccator, and chamber.

#### Ingredients used

The ingredients used in the research on the herb moonflower (*Tithonia diversifolia* (Hemsl.) Gray) were distilled water (CV. Rudang Jaya), Dimethylsulfoxide (Merck®), silica gel plate 60 GF254 (Merck®) and pro-analytical quality chemicals. (pa) Smart-Lab® output, ethylacetate. The media used in this study were Sabouraud Dextrose Agar/SDA (Merck®), Sabouraud Dextrose Broth/SDB (Merck®) media. Pure fungal cultures used were *Candida albicans* ATCC 10231 and *Microsporium canis*.

### **Plant moisture test**

Determination of water content is done by using the method of Thermogravimetric analysis. The tools used are porcelain dish, oven, and desiccator. How to determine rate:

A total of 3 g of simplicia herb flower moon powder was weighed, then dried in an oven at 100-105°C for 6 hours. The dried sample was then cooled in a desiccator and then weighed again. Then the difference in weight is calculated and the percentage of water content is calculated. % of water content is calculated with the formula: % water content =  $\frac{\text{final weight} - \text{initial weight}}{\text{sample weight}} \times 100\%$ .

### **Making simplicia and simplicia powder**

A total of 3.5 kg of moonflower herbs were cleaned of dirt by washing with running water, then drained, then dried in a drying cabinet at 40°C. The moonflower herb is considered dry when it is brittle (crushed into pieces), then the dried moonflower herb simplicia is placed in a blender and weighed by the powder, then stored in a tightly closed container, protected from sunlight and protected from moisture (Siti Nurjanah, Isbiyantoro, 2018).

### **Making Moonflower Herb Extract**

A total of 400 g of dried moonflower herb powder was put into a dark glass container, then macerated with 3 liters of 96% ethanol as a solvent. Covered, and stored at room temperature for 5 days protected from light while stirring occasionally, then filtered to obtain maserate (I). The pulp was macerated again with 1 liter of 96% ethanol for 2 days with the same procedure until the maserate (II) was obtained. Maserat I and Maserat II were combined, sorted and poured, then the result was evaporated using a rotary evaporator at a temperature of 50 °C to obtain a thick extract. Then the thick extract was put into a sterile container and tightly closed. The extract results were tested for chemical compound analysis, Thin Layer Chromatography (TLC) and antifungal activity tests.

### **2.3 Antifungal Activity Testing**

A total of 0.1 mL of *Candida albicans* ATCC 10231 and *Microsporium canis* inoculum were put into a sterile petri dish, then 35 mL of liquid SDA medium was poured into the cup and homogenized so that the media and fungal suspension were mixed and then allowed to stand for a while until solidified. Antifungal activity was tested using the well diffusion method using a metal buffer. The results of the metal backing molds that have solidified are in the form of wells, each with 0.1 mL of the test solution of the moonflower herb extract in various concentrations of 500 mg/mL, 400 mg/mL, 300 mg/mL, 200 mg/mL, 100 mg/mL and 50 mg/mL. As a negative control a mixture of DMSO and ethanol was used and as a positive control used nystatin drop of 100,000 IU/mL and fluconazole infusion of 2 mg/mL with the appropriate ratio used to dissolve the extract at each concentration then incubated at 25°C for 48 hours. The clear area around the well was observed and measured with a caliper. The treatment was repeated three times.

### 3. Result And Discussion

#### 3.1 Results of Identification of Plant Materials

The results of plant identification were carried out at the Herbarium Medanense (MEDA) University of North Sumatra, which stated that the plant studied was moonflower (*Tithonia diversifolia* (Hemsl.) Gray), family Asteraceae.

#### 3.2 The results of the Moisture Flower Herb's Moisture Test Results

The results of the water content test of the moon flower herb in the Pharmacognosy Laboratory of STIKes Arjuna, which stated that the moon flower herb had a water content of 10.2%.

#### 3.3 Results of TLC Examination of the Flower Moon Herb Extract

Table 1  
TLC Test Results of Kembang Bulan Herb Ethylacetate Extract

Mobile phase				
<i>n</i> -hexane: ethylacetate 7:3	Reactor	Rf Nilai Value	Color	Information
	Sulfuric acid		Yellow	
	50% in methanol		Green	
			Chocolate	
			Yellowish Green	
			Black	
Extract	Iron (III)	0,26	Yellow	
Ethylacetate	5% chloride	0,3	Brownish Green	
Herbs		0,5	Black	Polifenol
Flower		0,6	Green	Polifenol
Month	Liebermen-	0,3	Yellow	
	Bourchard	0,4	Green	
		0,5	Yellow	
	Dragendroff	0,2	Yellow	
		0,3	Yellowish Green	
		0,4	Yellow	
		0,5	Green	
		0,56	Chocolate	Alkaloid

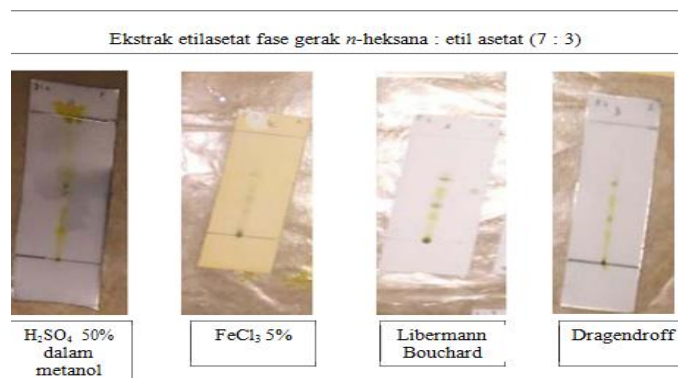


Figure 1

Thin Layer Chromatography Results of Ethylacetate Extract of Kembang Bulan Herb

Examination Result of Antifungal Activity Test of Flower Moon Herb Extract Against *Candida albicans* and *Microsporum canis*.

Table 2  
Ethylacetate Extract Inhibitory Diameter Measurement Results

Mold	Test	Concentration (mg/mL)						K+	K-
		500	400	300	200	100	50		
<i>Candida Albicans</i> ATCC 10231	1	11.5	10.5	10	0	0	0	15.5	0
	2	0	0	0	9	0	0	22.75	0
	3	11	10.95	9.75	0	0	0	15.8	0
	Average	7.5	7.15	6.58	3	0	0	18.01	0
<i>Microsporum canis</i>	1	21.65	20.45	16.5	0	0	0	21.5	0
	2	18.75	15.05	12.15	0	0	0	20.45	0
	3	20.35	16.65	11	0	0	0	20.5	0
	Average	20.25	17.38	13.21	0	0	0	20.81	0

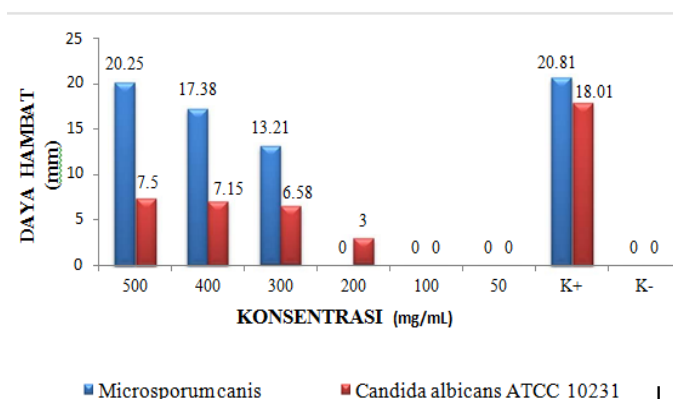


Figure 2  
Diagram Uji Aktivitas Antifungi Ekstrak Etilasetat

#### 4. Conclusion

Based on the results of the research that has been carried out, it can be concluded that the results of the thin layer chromatography (TLC) test with the mobile phase of n-hexane: ethylacetate (7:3) against the ethylacetate extract containing polyphenolic compounds, steroids and alkaloids, the results of the activity test of the ethylacetate extract of the moonflower herb ( *Tithonia diversifolia* (Hemsl.) Gray) was able to inhibit the growth of *Candida albicans* and *Microsporium canis* because the moonflower herb contains compounds that can interfere with the growth of these fungi and the minimum inhibitory concentration (MIC) of the ethylacetate extract of the moonflower herb against the fungus *Candida albicans* was at a concentration of 200 mg/mL, which was 3.00 mm, while the fungus *Microsporium canis* was found at a concentration of 300 mg/mL, which was 13.21 mm.

#### References

- Alfiah, R. R., Khotimah, S., & Turnip, M. (2015). Efektivitas Ekstrak Metanol Daun Sembung Rambat (*Mikania micrantha* Kunth) Terhadap Pertumbuhan Jamur *Candida albicans*. *Journal Protobiont*, 4(1), 52–57.
- Damayanti, R., & Ervilita, R. (2019). Skrining Fitokimia Ekstrak Etanol, Etil Asetat Dan N-Heksana Batang *myristica* *Fragrans*. *Talenta Conference Series: Science and Technology (ST)*, 2(1), 97–100. <https://doi.org/10.32734/st.v2i1.323>
- Fajri, M., Medison, I., Khairisyaf, O., & Russilawati, R. (2018). Efek Pemberian Antibiotika Terhadap Peningkatan Kolonisasi *Candida* Saluran Napas. *Jurnal Kesehatan Andalas*, 7(Supplement 4), 22. <https://doi.org/10.25077/jka.v7i0.944>
- Indarjulianto, S., Purnamaningsih, H., Wikansari, P., Yohanes Imanuel Sakan, G., Ilmu Penyakit Dalam, B., Farmakologi, B., Kedokteran Hewan, F., Gadjah Mada, U., Fauna No, J., Ugm, K., Malang, K., Studi Kesehatan Hewan, P., & Pertanian Negeri Kupang, P. (2014). Isolasi dan Identifikasi *Microsporium canis* dari Anjing Penderita Dermatofitosis di Yogyakarta (ISOLATION AND IDENTIFICATION OF *Microsporium Canis* FROM DERMATOPHYTOSIS DOGS IN YOGYAKARTA). 15(2), 212–216.
- Khatimah, K., Mone, I., & Fa'al Santri, N. (2018). Identifikasi Jamur *Candida* Sp Pada Kuku Jari Tangan Dan Kuku Kaki Petani Dusun Panaikang Desa Bontolohe Kecamatan Rilau Ale Kabupaten Bulukumba. *Jurnal Media Laboran*, 8(1), 39–43. <file:///C:/Users/USER/AppData/Local/Temp/387-Article Text-900-1-10-20190714-1.pdf>

- Ningsih, W., Firmansyah, F., & Anggraini, S. (2016). Formulasi Dan Uji Aktivitas Antibakteri Gel Pembersih Tangan Ekstrak Etanol Daun Kembang Bulan (*Tithonia Diversifolia* (Hemsley) a. Gray). *Jurnal Ilmiah Farmasi*, 12(2), 53–61. <https://doi.org/10.20885/jif.vol12.iss2.art6>
- Obafemi, C. A., Sulaimon, T. O., Akinpelu, D. A., & Olugbade, T. A. (2006). Antimicrobial activity of extracts and a germacranolidetype sesquiterpene lactone from *Tithonia diversifolia* leaf extract. *African Journal of Biotechnology*, 5(12), 1254–1258.
- Sasmita, F. W., Susetyarini, E., Husamah, H., & Pantiwati, Y. (2017). Efek Ekstrak Daun Kembang Bulan (*Tithonia diversifolia*) terhadap Kadar Glukosa Darah Tikus Wistar (*Rattus norvegicus*) yang Diinduksi Alloxan. *Biosfera*, 34(1), 22. <https://doi.org/10.20884/1.mib.2017.34.1.412>
- Siti Nurjanah, Isbiyantoro, H. F. (2018). EKSTRAK DAUN KEMBANG BULAN (*Tithonia diversifolia* (Hemsl.) A. Gray) SEBAGAI ANTIBAKTERI TERHADAP *Streptococcus mutans* dan *Streptococcus sanguinis*. 7(1), 33–40.
- Soedarto. (2015). Mikrobiologi kedokteran (Soedarto (ed.)). Sagung seto. <http://inlislite.uin-suska.ac.id/opac/detail-opac?id=3138>
- Sri Hartati Wahyuningsih, M., Afiyah Syarif, R., Suharmi, S., Murini, T., Saputra, F., & Suryo, A. W. (2013). Selectivity of Purified Extract From the Leaves of *Tithonia Diversifolia* (Hemsley) a.Gray) Against Hela Cells Selektivitas Ekstrak Terpurifikasi Daun *Tithonia Diversifolia* (Hemsley) a.Gray) Terhadap Sel Hela. *Traditional Medicine Journal*, 18(1), 2013.
- Utami, W.S., N. dan Y. A. (2012). Effect *Tithonia diversifolia* (( Hemley ) A . Gray ) Ethanol Extract as Antimalarial on Mice Strain BALB/C Before and After Infected by *Plasmodium berghei*. *Jurnal Medika Planta*, 1(5), 56–66.
- Widasmara, D., Suyoso, S., & Murtiastutik, D. (2014). Profil spesies *Candida* dari kandidiasis vulvovaginalis pada pasien HIV / AIDS yang mendapat antibiotik sistemik. *BIKK- Berkala Ilmu Kesehatan Kulit Dan Kelamin-Periodical of Dermatology and Venereology*, 26(3), 202–206.