Antifungal Activity of Pare Leaf (*Momordica charantia* L.) Methanol Extract

Baiq Naili Dewi Atika

Biologi, FMIPA, Universitas Nahdlatul Wathan Mataram, Mataram

**Email : nailyika16@gmail.com*

Abstract

Pare leaf (Momordica charantia L) is known to have potential as anti-bacterial and anti-fungal agent. This plant contains tannins, flavonoids, saponins, alkaloids, and triterpenoids. The administration of pare leaf methanol extract was hypothesized to increase inhibition zone in Candida albicans and Rhodotorulla sp colonies. The aim of this study was to determine antifungal activity of pare leaf methanol extract (PAME) against Candida albicans and Rhodotorulla sp. The method used in this study was well-diffusion method. Medium used for culture was MHA and each petri dishes was inoculated with suspension of either Candida albicans or Rhodotorulla sp., followed by making wells using sterilized straw. Pare leaf extract was added into each well at various concentration of 20%, 40%, 60%, 80%, 100%. Data was analyzed qualitatively based on diameter of inhibition zone to determine antifungal activity. Study was designed as experiment with completely randomized design. Result showed that the largest inhibitory zone was 12 mm at a concentration of 100% for inhibiting candida albicans while for inhibiting Rhodotorulla sp was 8 mm. Statistical test produced significant difference, indicating that pare (Momordica charantia L) leaf methanol extract was able to inhibit the growth of Candida albicans and Rhodotorulla sp.

Keywords: *antifungal, pare leaf, methanol extract*

INTRODUCTION

Candida albicans is the most prevalent fungal species isolated from oral, vulvovaginal and urinary tract. They are not only detected in immunocompromised patients, but also in 31–55% of healthy individuals (Samaranayake *et al*., 2002). Candida albicans can cause opportunistic infections from superficial mucous membrane lesions to life-threatening disease (Marable *et al.*, 2016), oral leukoplakia (Amer e*t al.*, 2017), Sjogren syndrome (Medeiros *et al.*, 2018), and oral squamous cell carcinoma (Bakri e*t al.*, 2010). These cases are harder to be cured if the lesions are infected by *Candida albicans*. *Candida albicans* will proliferate, increases virulence and turns into a pathogens. Moreover it caused infection called candidiasis. Meanwhile, *Rhodotorula* sp. has been implicated as the etiologic agent of central venous chateters infection, fungemial (Kiehn *et al.*, 1992), endocarditis, peritonitis, meningitis, and endophthalmitis (Tuon and Silvia, 2008). It was previously considered non-pathogenic, however in 2008 this yeast was fulfilled the criteria as an emerging pathogen (Tuon and Silvia, 2008).

Recently, the increased resistance of antifungal has attracted the attention of the scientific community. Fungi have become resistant to poliens, azoles and echinocandins, and the emergence of drug resistant strains has been reported in all fungal species (Robbins et al., 2017). The number of *Candida albicans*, non-albicans *Candida* sp., and *Rhodotorula* sp. resistant to the main antifungal agents has increased worldwide. Clinical and in vitro resistance to antifungal agents can be inherent to the microorganism itself, but also appears after previous

contact with the drug (Moraes *et al*., 2015). There has been an increasing search for new antifungal compounds due to the lack of efficacy, side effect and or resistence associated with some of the existing drugs (Intzar *et al*., 2010).

Pathogenic microorganisms have built great resistance against several synthetic antibiotics; as a result, much attention is being paid to isolate active compounds from plant species used in traditional medicine (Egharevba *et al*., 2019). Extracts from plants have been used since ancient times for the traditional treatment of diabetess, microbial infections, sources of antioxidant, etc. (Egharevba *et al.*, 2019).

The use of plant extracts as an alternative antifungal properties are based on the idea of secondary metabolic compounds from plants as a repellent and fungi inhibitor. One of the plants that have antifungal compound is Pare (*Momordica charantia* L.). Pare is known contained Glycoside kukurbitasin type momordicine, alkaloids, flavonoids, saponins and steroid/triterpenoid. Most studies of antifungal activity of pare extract have been widely used in *Staphylococcus aureus, E. coli* (Jagessar *et al*., 2008), and *Trypanosoma cruzi* (Santos *et al*., 2012). However, the study on antifungal activity of pare leaf methanol extract (PAME) was yet to be explored. This study was aimed to confirm PAME as antifungal and antiseptic agents and evaluate this inhibitory action as different extract concentration against *Candida albicans* and *Rhodotorula* sp.

METHODS

Plant Filtrates Preparation

Pare leaf were grinded to powder using a blender. Ten one gram of PAME was weighed in an Erlenmeyer and dissolved in 100 ml of distilled water (v:v). It added to pre-filtrate. The Erlenmeyer is placed in dark for three days in room temperature. The mixture was filtered using Whitman No. 1 filter paper. The filtrates were kept at 4^{o}C until use.

Microorganisms

Two reference fungi, Candida albicans and Rhodotorulla sp, were used during the study. The tested strains were obtained from the clinic hospitals NTB, cultured in nutrient broth at 37^{o}C and stored in nutrient agar slants at 4^{o}C.

Antifungal Assay

The antimicrobial potential of PAME was tested using a well diffusion assay. Fungal strains grown on muller hinton agar at 37 ^{o}C for 24 h were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standards (10^{8} CFU/ml). The suspension 1000 µl was used to inoculate in petri plates. The dissolution of the plant filtrates was facilitated with the addition of 1% (v/v). Briefly the muller hinton agar medium (25 mL) was poured into Petri dishes under aseptic conditions in a laminar flow hood. The plates were kept in the laminar flow chamber for solidification of the media. The plates were then kept in laminar flow for drying. Once dried, Make a well using a syringes in the plate and add of the test solution on each disk.

The diameters of inhibition zones were measured in mm after incubation at 37°C for 24 h. All experiments were in quadruplicate for each treatment against each fungal.

RESULT AND DISCUSSION

This study was designed to investigate the antifungal activity of PAME. The antifungal activity of PAME was qualitatively assessed by the presence or absence of inhibition zones and the results are depicted in (Table 1).

Table 1. Antifungal activity of extract pare leaf against *Candida albicans* and *Rhodotorula* sp.

	Concentration of PAME	Inhibition zone diameter (mm)			
		I	II	III	IV
Rhodotorula sp.	20 %	8	8	8	8
	40%	8	10	10	10
	60 %	11	11	10	10
	80%	10	10	10	10
	100%	10	12	12	12
Candida albicans	20 %	6	5	3	0
	40%	8	8	6	0
	60 %	8	7	9	10
	80%	10	9	9	10
	100%	10	10	12	12

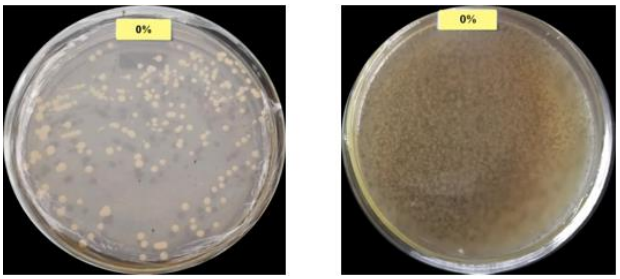

Fig. 1. *Candida albicans* (left) and *Rhodotorula* sp. (right).

Pare (*Momordica charantia* l.) is one of the traditional medicinal plants known to have some of the chemical content such as momordicin, momordioksida, quarantine, resin acid, trikosanik acid, resinat, alkaloids, flavonoids, saponins, tertreponoid, vitamins A and C, as well as fatty oils consisting of oleic acid, linoleic acid. According to this study (Table 1), it showed that PAME has the ability as an antifungal against *Candida albicans* and *Rhodotorula* sp. The data showed that various concentration of PAME can inhibit the growth of *Candida albicans* and *Rhodotorula* sp.

Table 1. showed that inhibition zones of PAME ranged from the higher concentration that (20%) with diameter of 8 mm and 3 mm compare with the control group (Fig. 2). The diameter of the zones at a concentration of 20% was 5 ml. The biggest inhibitory zones are present in the concentration of 100% with diameter of 12 mm (Fig. 1). Our results showed that PAME was able to inhibit the growth of *Candida albicans* and *Rhodotorula* sp. The effect of PAME was

stronger to inhibit the growth of *Rhodotorula* sp than *Candida albicans*. It is because *Candida albicans* could forms surface-attached microbial communities known as biofilms (Tulasidas et al., 2018). This biofilm will further develop to form a microenvironment for microorganisms aimed to preserving life. Cells in biofilms is different with planktonic cells, which are more resistant to conventional antifungal drugs (Ramage et al., 2001).

In addition, growth inhibition of these fungus can be assumed to inhibition of cell wall synthesis against the compounds contained in the PAME. The cell walls of microbes have a very important role as the outermost components of microbes that interact directly with the environment in defending itself against threats from the environment. The cell wall is able to maintain the microbial forms and as a protector of the cells. The main components of the *Candida albicans* cell wall are proteins and polysaccharides (Chaffin, 2008), which assumed to have higher protection than *Rhodotorula* sp.

The ability of PAME to inhibit the growth of fungi depends on the concentration of active compounds dissolved in the extract. Pare leaf extract contained kukurbitasin, glycosides, momordicine. Alkaloid compounds, saponins, flavonoids and steroids/triterpenoids are identified in the screening of pare leaf extract. Flavonoids are compounds that have pharmacological effects as antifungals. Flavonoids has the ability to form complexes with proteins and damage cell membranes by denaturing protein bonds in the cell membrane, it caused cell membranes lysis. Futhermore these compounds can penetrate into the nucleusand caused DNA damage. In addition, the mechanism of inhibiting microbial growth is by interrupt the activity of cytoplasmic membrane, including disturb the active transport in cells. Meanwhile, saponins play an important role in reducing cell surface tension. It caused the cell walls damaged and leakage in microbial cell membranes (Wankhede, et al., 2013).

In general, the inhibitory action of bacterial or fungal growth by PAME is closely related to the ability of secondary metabolites which able to disturb the cell wall and penetrate the fungal cell membrane. It disrupt the proton acid-base balance and energy production in the fungal cells. Thus in this study, PAME showed the ability to inhibit the growth of *Rhodotorula* sp. and *Candida albicans*.

CONCLUSION

Based on microbial detection assay, this study showed the variation of pare leaf (*Momordica charantia* l.) Methanol extract has the ability to inhibit the growth of *Rhodotorula* sp. and *Candida albicans.*

REFERENCES

Amer, S. Galvin, C.M. Healy, G.P. Moran. (2017). The microbiome of potentially malignant oral leukoplakia exhibits enrichment for Fusobacterium, Leptotrichia, Campylobacter, and Rothia species, Front. *Microbiol*. 8:2391. https://doi.org/10.3389/fmicb.2017.02391

Bakri, H. Mohd Hussaini, A. Rachel Holmes, R. David Cannon, A. Mary Rich. (2010). Revisiting the association between candidal infection and carcinoma, particularly oral squamous cell carcinoma, *J. Oral Microbiol*. 2. https://doi.org/10.3402/jom.v2i0.5780

C. Medeiros, L.G. Dos Anjos Borges, K. Cherubini, F.G. Salum, Medina da Silva R, de Figueiredo M. (2018). Oral yeast colonization in patients with primary and secondary Sjögren's syndrome, Oral Dis. 24:7: 1367–1378. https://doi.org/10.1111/odi.12896

Chaffin, W.L. (2008). *Candida albicans* Cell Wall Proteins. *Journal of American Society for Microbiology*. 72: 3: 495-544. https://doi.org/10.1128/MMBR.00032-07

D.R. Marable, L.M. Bowers, T.L. Stout. (2016). Oral candidiasis following steroid therapy for oral lichen planus, Oral Dis. 22: 2: 140–147. https://doi.org/10.1111/odi.12399

D.S. Perlin, R. Rautemaa-Richardson, A. Alastruey-Izquierdo. (2017). The global problem of antifungal resistance: prevalence, mechanisms, and management, Lancet Infect. Dis. 17: 12: e383–e392. https://doi.org/10.1016/S1473-3099(17)30316-X

Egharevba, O.O. Dosunu, S.O. Oguntoye, N.S. Njinga, H.A. Abdulmumeen, M.B.A. Adebayo. (2018). Phytochemical screening, antimicrobial and antioxidant activities of the crude extract of Senecio abyssinicus flower, *J. Pharmaceut. Res. Develop*. Pract. 2: 1: 16–24. https://doi.org/10.13140/RG.2.2.27117.64486

Egharevba God'shelp, Omotayo O. Dosumu, N.S. Njinga, Steven O. Oguntoye, H. Amao Abdulmumeen, Oluyori P. Abimbola, Ashok K. Tiwari, Ajay Anand, Amtul Zehra, U. Priyanka. (2019). Phytochemical constituents, antioxidant and antimicrobial activities of extracts of Tephrosia bracteolata leaves, in: *International Conference of Science and Sustainable Development.* ICSSD. https://doi.org/10.1089/jmf.2015.0099

G. Ramage, K. Vande Walle, B.L. Wickes, J.L. López-Ribot. (2001). Standardized method for in vitro antifungal susceptibility testing of Candida albicans biofilms, Antimicrob. Agents Chemother. 45:9: 2475-2479. https://doi.org/10.1128/aac.45.9.2475-2479.2001

Intzar A, Farrah GK, Krishan AS, Bishan GD, Naresh KS, Prabhu D. (2010). In vitro antifungal activity of hydroxychavicol isolated from Piper betle L. Ann Clin Microbiol Antimicrob. 9:7. https://doi.org/10.1186/1476-0711-9-7

Jagessar R.C., A. Mohammed, and G. Gomes. (2008). An evaluation of the Antibacterial and Antifungal activity of leaf extracts of Momordica Charantia against Candida albicans, Staphylococcus aureus and Escherichia coli. Nature and Science. 6(1), ISSN: 1545-0740

J. Chandra, P.K. Mukherjee, S.D. Leidich. (2001). Antifungal resistance of candida biofilms formed on denture acrylic in vitro, J. Dent. Res. 80: 3: 903–908. https://doi.org/10.1177/00220345010800031101

Keihn TE, Gorey E, Brown AE, Edwards FE, Amstrong D. (1992). Sepsis due to Rhodotorula related to use of indwelling central venous catheters. Clin Infect Dis. 14: 841-846. https://www.jstor.org/stable/4456419

Khan, A. M., (2011). Phytochemical Analysis of Selected Medicinal Plants Of Margalla Hillsm And Surroundings. Journal of Medicinal Plants Research 5: 25: 6017-6023. https://doi.org/10.5897/JMPR11.869

Moraes, R.C., Lana, A.J.D., Kaiser, S., Carvalho, A.R., de Oliveira, L.F.S., Fuentefria, A.M., Ortega, G.G., (2015). Antifungal activity of Uncaria tomentosa (Willd.) D.C. against resistant non-albicans Candida isolates. Ind. Crops Prod. 69, 7–14. https://doi.org/10.1016/j.indcrop.2015.01.033

Robbins, N., Caplan, T., Cowen, L.E., (2017). Molecular evolution of antifungal drug resistance. Annu. Rev. Microbiol. 71 (1), 753–1775. https://doi.org/10.1146/annurevmicro-030117-020345.

Samaranayake, L.P., Fidel, P.L., Sweet, S.P., Teanpaisan, R., Coogan, M.M., Blignaut, E., Wanzala, P.,

(20020. Fungal infections associated with HIV infection. Oral Dis. 8, 151–160. https://doi.org/10.1016/S1473-3099(17)30303-1

Santos KK, Matias EF, Sobral-Souza CE, Tintino SR, Morais-Braga MF, Guedes GM, Santos FA, Sousa AC, Rolón M, Vega C, de Arias AR, Costa JG, Menezes IR, Coutinho HD. (20120. Trypanocide, cytotoxic, and antifungal activities of Momordica charantia. Pharm Biol. 50(2): 162-166. https://doi.org/10.3109/13880209.2011.581672

Tulasidas S, P. Rao, S. Bhat, R. Manipura. (20180. A study on biofilm production and antifungal drug resistance among Candida species from vulvovaginal and bloodstream infections, Infect. Drug Resist. 11: 2443–2448. https://doi.org/10.2147/IDR.S179462

Tuon F. F., and Silvia F.C. (20080. Rhodotorula infection. A systematic review of 128 cases from literature. Rev Iberoam Micol; 25: 135-140. https://doi.org/10.1016/S1130-1406(08)70032-9

Wankhede, S.B., Madhushree Madhumita Routh, Sandeep Balsingh Rajput, Sankuny Mohan Karuppayil. (2013). Antifungal Properties of Selected Plants of Apocynaceae Family against the Human Fungal Pathogen *Candida albicans.* International Current Pharmaceutical Journal. 2: 7. https://doi.org/10.3329/icpj.v2i7.15155