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CHEMICAL COMPOSITION AND ANTIFUNGAL ACTIVITY OF *Morinda Citrifolia* FRUIT EXTRACT

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Abstract

Noni (*Morinda citrifolia*) fruit is a well-known plant used as a traditional medicine for preventing some diseases because of its abundance in chemical compounds. This research aimed to determine the phytochemical concentration, chemical composition, and antifungal activity of *M. citrifolia* fruit extract. *M. citrifolia* fruit was extracted with methanol and then distilled water for the partition extract. Subsequently, the extract was fractionated using various nonpolar to polar solutions, such as; chloroform, ethyl acetate, water, 2-propanol, and methanol fractions. Each fraction was evaporated until the dry extract was released. Additionally, the photochemical concentration of the *M. citrifolia* fruit extract was quantitatively determined using a UV-visible spectrophotometer. The chemical composition of the *M. citrifolia* fruit extract of each fraction was identified using gas chromatography-mass spectrometry (GC-MS). Then, the antifungal activity of *M.citrifolia* fruit extract against *C. albicans* and *C. krusei* was determined using the disc diffusion method. The results showed that the phytochemical concentration of the *M. citrifolia* fruit extract was 1970.25 ppm flavonoids, 35.61 ppm tannins, and 148.62 ppm steroids. 2-Fluorobenzoic acid, eucalyptol, 2-chloroaniline-5-sulfonic acid, hexa-decamethyl octasiloxane, and tetra-propyl stannane were found to be the major components of *M. citrifolia* fruit extract. According to the research, *M. citrifolia* fruit extract showed antifungal activity against *C. albicans* and *C. krusei* in all tested fractions. The maximum inhibition zone of *C. albicans* was 14.0 ± 1.00 mm in the 2-propanol fraction, while that of *C. krusei* was 11.7 ± 0.58 mm in the methanol fraction.

Keywords: Antifungal activity. *C. albicans*. *C. krusei*. *M. citrifolia* fruit.

1. Introduction

Th noni (*Morinda citrifolia*) plant is a well-known plant used as a traditional medicine for some diseases. It is used as a traditional medicine because almost all parts of the *M. citrifolia* plant have the potential to prevent disease. According to Ristoja program, the *Battra* ethnics group living in Meranjat Village, Ogan Ilir South Sumatera Province, Indonesia, uses *M. citrifolia* fruit as medicine (Kemenkes 2017).

All parts of *M. citrifolia* have benefits for preventing various diseases such as cancer, infection,

arthritis, diabetes, asthma, hypertension, and pain (Wang et al. 2002; Algenstaedt et al. 2018). In addition, this fruit is helpful as a folk medicine for the prevention of dysentery, heartburn, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, intestinal digestion, heart atherosclerosis, blood circulation problems, and drug addiction (Siddiqui et al. 2008; Ali et al. 2016; Yee, 2019). Furthermore, previous studies reported that *M. citrifolia* has antimicrobial, anticancer, antioxidant, anti-inflammatory, analgesic and cardiovascular activities (Nayak et al. 2015; Senthilkumar et al. 2016; Abou Assi et al. 2017). The methanol extract from the *M. citrifolia* fruit has an anti-proliferation effect (Hermansyah and Susilawati 2017). *M. citrifolia* fruit contains phytochemicals such as phytoestrogens, oligosaccharides, polysaccharides, flavonoids, phenols, asperulosides, iridoids, esters, fatty acids, and scopoletin, which have antibiotic activity, and catechin, epicatechin, beta-sitosterol, and damnacantha, which are protein inhibitors of HIV (Senthilkumar et al. 2016).

Candidiasis vaginalis is an infection that affects the the reproductive system of women. Almost 70% of women will be infected by candidiasis vaginalis during their lifetime and more than 10% of those women will be attacked again by C. spp. more than once (Weissenbacher et al. 2009; Hermansyah et al. 2017). The impact of *C. spp.* on the reproductive system of women is a serious problem, and *C.* spp can infect the vagina and cause vaginal discharge and whiteness. Previous research investigated *C. spp.* in women infected by candidiasis vaginalis using the multiplex PCR method. This study found that *C. krusei* has a sensitivity of 100%, specificity of 61.1%, positive prediction value of 63.2%, and negative prediction value of 100%. Meanwhile, *C. albicans* has a sensitivity of 33.3%, specificity of 100%, positive prediction value of 100%, negatively prediction value of 93.1% (Susilawati et al. 2019).

Candida is a unicellular cell (*yeast* or *yeast-like*) consisting of 150 species, but only 17 species have been reported to infect humans. The common species that cause vulvuvaginitis are *C. albicans, C. glabrata, C. tropicalis, C. krusei, C. stellatoidea,* and *C. parapsilosis* (Taher 2009; Hermansyah et al. 2017; Susilawati et al. 2019). However, the species that commonly infect humans are *C. albicans* (approximately 70-80%) and *C. tropicalis* (approximately 30-40%) (Wahyuningsih et al. 2012).

Therefore, the objectives of this study were to investigate the phytochemical concentration, chemical composition, and antifungal activity of *M. citrifolia* fruit extract. *M. citrifolia* fruit was extracted with methanol and then distilled water for the partition extract. The extract was fractionated using various non-polar to polar solutions such as; chloroform, ethyl acetate, water, 2-propanol, and methanol fractions. Subsequently, the antifungal activity of the *M. citrifolia* fruit extract against *C. albicans* and *C. krusei* was determined using the disc diffusion method. At the same time, the chemical compositions of each fraction of *M. citrifolia* fruit extract were also investigated using GC/MS.

2. Material and Methods

Preparation of *M. citrifolia* fruit extract

Fresh *M. citrifolia* fruit was collected from Tebedak to Payamaran Village, South Sumatera Province, Indonesia. A fresh fruit sample was washed to remove dust and other impurities. The sample was weighed and sliced to obtain a dried sample and ground into 60 mesh. Furthermore, a 1000 g sample of *M. citrifolia* fruit was extracted using methanol (5 X 1 L) for 24 hours. *M. citrifolia* fruit extract was then evaporated to obtain the pasta. The pasta was then extracted with distilled water for the partition extract. Subsequently, the extract was fractionated using various non-polar to polar solutions such as; chloroform, ethyl acetate, water, 2-propanol, and methanol fractions. Each fraction was evaporated until the dry extract was released.

Quantitative assay of phytochemicals in *M. citrifolia* fruit extract

Quantitative assays of phytochemicals such as flavonoids, steroids, and tannins were conducted with minor modifications according to Pratiwi et al. (2021), Ncube et al. (2011), and Selvakumar et al (2019), respectively.

The steroid content in the extract was measured by the photometric method using prednisone as

a standard. A 1.0 mL sample was added to 2 mL ethyl acetic, 1.0 mL anisaldehyde-ethyl acetic, and 1.0 mL sulfuric acid-ethyl acetic, and the solution was incubated in a water bath for 20 minutes. The absorbance of the solution was measured using a spectrophotometer at λmax 400 nm.

The total tannin content was determined using Folin-Ciocalteu method. Approximately 0.1mL of noni extract was added to 7.5 mL distilled water, 0.5 mL Folin-Ciocalteu phenol reagent, and 1 mL of 35% Na_2CO_3 solution and diluted to 10 mL with distilled water. After incubating at 30 °C with shaking for 30 min, the absorbance of the solution was measured using a UV-Visible spectrophotometer at λmax 725 nm, and gallic acid was used as standard.

The flavonoid content was determined by spectrophotometry. Approximately 1 mL diluted sample and standard was added to 0.3 mL of 5% NaNO2 solution, mixed thoroughly, and incubated for 5 min. Approximately 0.3 mL of 10% $AlCl_3$ was added, and the mixture solution was measured by spectrophotometer at λmax 510 nm.

Analysis chemical compound of *M. citrifolia* fruit extract

The *M. citrifolia* fruit extract of each fraction was identified using gas chromatography-mass spectrometry (GC-MS).

Research design

This research was an experiment performed using a posttest and control group design. The group was divided into the *C. albicans* group and the *C. krusei* group. Each group consists of five fractions and one control:

1. Ketokonazole as a control
2. Chloroform fraction (0, 250, 500, and 1000 µg/mL)
3. Ethyl acetate fraction (0, 250, 500, and 1000 µg/mL)
4. Water (0, 250, 500, and 1000 µg/mL)
5. 2-propanol(0, 250, 500, and 1000 µg/mL)
6. Methanol (0, 250, 500, 1000 µg/mL)

To determine a minimum inhibition concentration, 9 serial concentrations were used (3.9, 7.81, 15.625, 31.23, 62.5, 125, 250, 500, and 1000 µg/mL). A minimum inhibitory concentration (MIC) assay was conducted according to Ramschie et al. (2017) with slight modification.

Preparation of *C. albicans* and *C. krusei*

C. albicans was obtained from the Pharmacy Laboratory of Institute Teknologi Bandung, while *C. krusei* was obtained from the Parasitology Laboratory Faculty of Medicine Universitas Indonesia. *C. albicans* and *C. krusei* were regenerated by culturing in Sabouraud agar to obtain a single colony, that was pure and stable. Single colonies of each fungus were inoculated in 0.5 mL of broth heart infusion (BHI) and incubated at 37 °C for 24 hours. The suspensions were adjusted by the standard method of 0.5 McFarland for 1.10^8 CFU/mL (Suryaningsih et al. 2015).

Antifungal activity assay of *C. albicans* and *C. krusei*

The activity of the *M. citrifolia* fruit extract was determined using the agar disc diffusion method. Then 100 µl suspensions of *C. albicans* and *C. krusei* were spread on Sabouraud agar media and incubated at 37 °C for 2 x 24 hours. Subsequently, disc paper was impregnated with *M. citrifolia* fruit extract from fractions of various concentrations namely, 0, 250, 500, 750 and, 1000 µg/mL. The disc papers were then placed aseptically on the surface of agar plates. Furthermore, the plates were incubated at 37 °C for 2-3 days and the diameter of the inhibition areas was measured in millimeters (Barani et al. 2014).

Statistical analysis

Data are displayed in triplicate to obtain a valid statistical evaluation of the result. All results represent the mean ± SD and were analyzed using a T test with a significance level of 0.05.

3. Results

Phytochemical properties of *M. citrifolia* fruit extract

The quantitative analysis of phytochemical compounds was determined using the linear regression curve of the standard solution. The flavonoid concentration was calculated using the regression equation y = 0.002x + 0.2075, the regression equation for tannin was y = 0.0108x – 0.0634, and steroid were calculated using the regression equation y = 0.0001x + 0.0986. Furthermore, the curve of each standard solution was applied to obtain the phytochemical concentration of the *M. citrifolia* fruit extract. Table 1 shows that the concentration of flavonoids, tannins and steroid was was 1970.25 ppm, 35.61 ppm, and 148.62 ppm, respectively.

Table 1. Concentrations of phytochemical compounds in *Morinda citrifolia* fruit extract.

No	Chemical compound	Concentration (ppm)
1.	Flavonoid	1970.25
2.	Tannin	35.61
3.	Steroid	148,62

Chemical properties of *M. citrifolia* fruit extract

The chromatographic analysis of the *M. citrifolia* fruit extract using GC-MS successfully identified 32 compounds (chromatogram data not shown). The chemical compounds in the *M. citrifolia* fruit extract are shown in Table 2. The major components were 2-fluorobenzoic acid (44.41%) and eucalyptol (31.70%) in the chloroform fraction, eucalyptol (41.64%) and 2-chloroaniline-5-sulfonic acid (23.15%) in the ethyl acetate fraction, eucalyptol (13.08%) in the water fraction, hexadecamethyl octasiloxane in the 2-propanol fraction, and tetrapropyl stannane (31.06%) in the methanol fraction.

Table 2. Chemical composition of *Morinda citrifolia* fruit extract.

No.	Chemical compounds	tr (min)	Peak area (%)
	Chloroform fraction		
1	1-Methyl-4-(1-methylethyl) benzene	4.85	1.80
2	Eucalyptol	4.95	31.70
3	5-(Hydroxymethyl)-2-Furancarboxaldehyde 2-propyl Thiophene	6.96	2.98
4	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan 2-Fluorobenzoic acid	7.68	44.41
5	Phenol, 2,4-bis(1,1-dimethylethyl)	9.73	2.37
6	2-Ethylacridine	17.21	4.01
7	Hexamethyl Cyclotrisiloxane	20.62	4.03
8	3,5-bis(1,1-Dimethylethyl)-1,2-benzenediol	23.08	5.68
9	Trimethyl-silane	25.47	3.02
	Ethyl acetate fraction		
1	2-Cyclopentene-1,4-dione	3.38	1.29
2	3-Methyl pentanoic acid, Hexanoic acid, methyl ester	3.73	1.82
3	1-methyl-2-(1-methylethyl)benzene 1-methyl-4-(1-methylethyl)benzene	4.85	2.53
4	Eucalyptol	4.95	41.64
5	Octanoic acid, methyl ester 4-Amino-2-methyl-2H-pyrazole-3-carboxylic acid	5.86	4.27

Table 2. Continued.

6	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan 3-methyl-thiophene-2-carboxamide	7.67	2.52
7	(+-)-5-(1-Acetoxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one semicarbazone	15.56	9.06
8	2-Chloroaniline-5-sulfonic acid	18.80	23.15
9	1-Methyl-2-phenyl-1H-Indole	19.10	10.00
	Water fraction		
1	Eucalyptol	4.94	13.08
2	1,1,3,3,5,5,7,7,9,9,11,11,13,13-Tetradecamethyl heptasiloxane	15.16	0.63
3	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl octasiloxane	15.46	0.74
4	1-(4,7-Dihydro-2-methyl-7-oxopyrazolo*1,5-a+pyrimidin-5-yl)-formic acid, methyl ester	15.80	1.72
5	Hexamethyl cyclotrisiloxane	15.85	1.21
6	1,1,3,3,5,5,7,7,9,9,11,11,13,13-Tetradecamethyl heptasiloxane	16.09	3.98
7	3,5-Bis(1,1-dimethylethyl)-1,2-benzenediol,	16.31	4.54
8	decamethyl tetrasiloxane	17.57	2.33
9	N-Methyl-1-adamantaneacetamide	18.07	3.04
10	2,4-Dimethyl Benzo*h+quinoline	18.24	0.79
11	Methyltris(trimethylsiloxy)silane	20.11	1.25
12	Trimethyl*4-(2-methyl-4-oxo-2-pentyl)phenoxy+silane	21.63	1.01
13	2,2,5a-Trimethyl-1a-*3-oxo-1-butenyl+ perhydro-1-benzazirene-1- carboxylic acid, -, methyl ester	22.06	0.43
14	Silicic acid, diethyl bis(trimethylsilyl) ester	25.60	0.80
	2-Propanol fraction		
1	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl octasiloxane tetradecamethyl cycloheptasiloxane 2-Benzo*1,3+dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	9.41	100.00
	Methanol fraction		
1	(+-)-5-(1-Acetoxy-1-methylethyl)-2-methyl-2-cyclohexen-1-one semicar bazone tetrapropyl stannane 3,5-bis-trimethylsilyl-2,4,6-cycloheptatrien-1-one 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl octasiloxane	18.92	31.06
2	1-Methyl-3-phenylindole 2'-(trimethylsiloxy)-Propiophenone	19.32	18.03
3	5-Methyl-2-phenyl-1H-Indole decamethyl Tetrasiloxane 5-Methyl-2-phenylindolizine	20.92	18.65
4	9,10-Dihydro-9,9,10-trimethyl anthracene 1-methyl-2-phenyl-1H-indole 1,1,3,3,5,5,7,7,9,9,11,11,13,13-Tetradecamethyl heptasiloxane	21.90	15.14
5	1,4-Dihydro-5-cyano-2-hydroxy-4-(4-isopropylphenyl)-6-methyl-, ethyl esterPyri- dine-3-carboxylic acid	22.21	17.12

Disc diffusion assay

The antifungal activity of each extract fraction of *M. citrifolia* fruit was measured using the disc diffusion method (Figure 1) and determined according to the inhibitor areas based on the concentration of each fraction. Furthermore, the antifungal activity of *M. citrifolia* fruit against *C. albicans* ranged between 6.3 ± 0.58 mm and 14.0 ± 1.00 mm, while that against *C. krusei* ranged between 5.7 ± 1.15 mm and 11.7 ± 0.58 mm as shown in Table 3.

The maximum antifungal activity against *C. albicans* was at a concentration of 1000 ppm in the 2-propanol fraction (14.0 ± 1.00 mm), followed by the methanol fraction (12.0 ± 1.73 mm), ethyl acetate fraction (12.0 ± 1.73 mm), chloroform fraction (9.7 ± 2.08 mm), and water fraction (9.3 ± 0.58 mm). Meanwhile, the maximum antifungal activity against *C. krusei* was at a concentration of 1000 ppm in the methanol fraction (11.7 ± 0.58 mm), followed by the chloroform fraction (10.0 ± 1.00 mm), 2-propanol

fraction (9.7 ± 0.58 mm), ethyl acetate fraction (9.3 ± 1.53 mm), and water fraction (750 ppm,8.7 ± 1.53 mm). According to the results of the disc diffusion method, *M. citrifolia* fruit extract showed an antifungal effect against *C. albicans* and *C. krusei* et all tested fractions. Based on Davis and Stout's criteria, the ability of *M. citrifolia* fruit extract was strong and moderate. The antifungal activity of the *M. citrifolia* fruit extract against *C. albicans* was shown to be higher than that against *C. krusei*. Furthermore, the antifungal activity of the *M. citrifolia* fruit extract showed less inhibition than the ketokonazole used as a positive control.

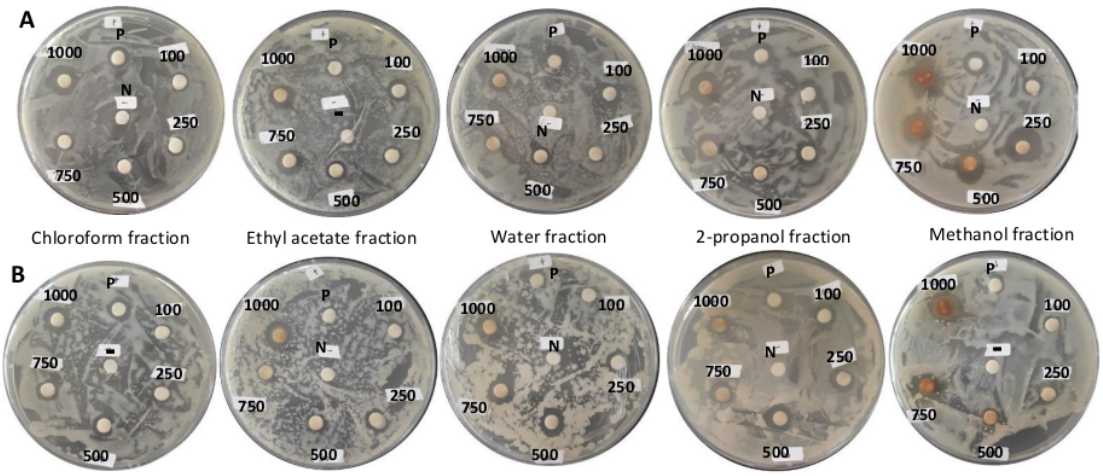

Figure 1. Inhibition zone of each extract fraction of Morinda citrifolia fruit against A - Candida albicans and B - Candida krusei. (Counterclockwise: P: positive control, 1000 ppm, 750 ppm, 500 ppm, 250 ppm, 100 ppm, N: negative control).

Table 3. Antifungal activity of each extract fraction of *Morinda citrifolia* fruit against *Candida albicans* and *Candida krusei*.

Fraction	Concentration (ppm)	Antifungal activity (mm)	
		Candida albicans	*Candida krusei*
Chloroform	100	6.3 ± 0.58	6.3 ± 0.58
	250	6.3 ± 0.58	7.3 ± 0.58
	500	6.3 ± 0.58	6.7 ± 1.15
	750	7.5 ± 0.50	8.7 ± 0.58
	1000	9.7 ± 2.08	10.0 ± 1.00
Ethyl acetate	100	7.3 ± 0.58	6.0 ± 1.00
	250	7.0 ± 1.00	6.0 ± 1.00
	500	9.0 ± 0.00	6.7 ± 0.58
	750	10.3 ± 0.58	6.3 ± 0.58
	1000	12.0 ± 1.73	9.3 ± 1.53
Water	100	6.3 ± 1.53	5.7 ± 1.15
	250	6.3 ± 1.15	7.3 ± 0.58
	500	8.0 ± 1.00	7.7 ± 1.53
	750	7.3 ± 0.58	8.7 ± 1.53
	1000	9.3 ± 0.58	8.3 ± 2.08
2-propanol	100	7.3 ± 1.15	8.7 ± 2.08
	250	7.0 ± 2.00	7.3 ± 1.15
	500	9.0 ± 1.73	9.0 ± 1.00
	750	9.3 ± 1.15	8.3 ± 0.58
	1000	14.0 ± 1.00	9.7 ± 0.58
Methanol	100	7.7 ± 0,58	7.3 ± 0.58
	250	8.0 ± 2,00	7.2 ± 0.29
	500	9.3 ± 2,31	8.0 ± 1.00
	750	11.0 ± 1,00	8.2 ± 0.29
	1000	12.0 ± 1,73	11.7 ± 0.58
Ketokenazole	500	11.3 ± 0.58	12.0 ± 1.00

Based on ANOVA, inhibition growth zones of *C.krusei* and *C.albicans* showed a highly significant difference between each concentration group, and the value was 0 ($p < 0.05$), indicating significant difference between each concentration. The F tests were 7.030 and 8.520, respectively, while the F-table was 6.95. Thus, F test > F-table indicated that different concentrations were significantly different from the inhibition zone.

4. Discussion

The phytochemical properties of *M. citrifolia* fruit extract contained flavonoids (such as flavones, flavonols, anthocyanidins, flavanols, flavanones, flavanonols, aurones, furan chromones, isoflavones, isoflavononones, biflavones, xanthones, chaocones, and dihydrochalcones), tannins, and steroids (such as stigma sterol, daucosterol, and β-sitosterol), similar to previous reports (Nagalingam et al. 2012; Afiff and Amilah 2017; Youn and Chang 2017; Sogandi and Nilasari 2019; Yee 2019; Ayunda et al. 2020). This study showed that the major components of *M. citrifolia* fruit extract were 2-fluorobenzoic acid, eucalyptol, 2-chloroaniline-5-sulfonic acid, hexadecamethyl octasiloxane, and tetrapropyl stannane. In agreement with our study, chemical compounds of octanoic and hexanoic acids constituted a major component of *M. citrifolia* fruit extracts (38.7% and 20.0%, respectively) (Holanda et al. 2020). These chemical constituents were found to be less abundant in our research. This condition may be due to some factors, such as the cultivation condition of the plant, the location of growth, and the extraction technique.

M. citrifolia fruit extract is known to have antimicrobial activity against viruses, bacteria, and fungi. Furthermore, the recent results showed that *M. citrifolia* fruit extract moderately inhibited the growth of *C. albicans* and *C. krusei*. Previous research reported that *M. citrifolia* fruit extract strongly inhibited the growth of *C. albicans* (16.6 ± 0.3 mm) (Afiff and Amilah 2017). In addition *M. citrifolia* leaf extract inhibited the growth of *Staphylococcus aureus* (12 mm), *Pseudomonas aeruginosa* (11 mm), and *Bacillus subtilis* (7 mm) (Nayak et al. 2015).

Using cultures, the growth of *C. albicans* was not detected with 50 mg/mL extract at 30 minutes of contact time or with 60 mg/mL extract at 15 minutes of contact time. According the broth dilution test, the minimum fungicidal concentration of the extract against *C. albicans* was 40 mg/mL at 90 minutes of contact time or 50 mg/mL at 15 minutes of contact time. (Jainkittivong et al. 2009).

M. citrifolia extract at 1000 µg/ml effectively inhibited the growth of *C. albicans* (16.6 ± 0.3) compared with the positive control, amphotericin B (20.6 ± 0.6). It was found to be a dose-dependent reaction (Barani et al. 2014).

This review examined azole resistance in infections caused by C. albicans as well as the emerging non albicans Candida species *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. glabrata* and in particular, describes the current understanding of the molecular basis of azole resistance in these fungal species. Although Candida species generally cause fungal infections in humans, some intrinsic azole resistance in some Candida species as well as the development of high-level azole resistance is a problem of critical importance in the clinical setting (Whaley et al. 2017). Azole resistance has occurred in infection caused by *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. glabrata* (Whaley et al. 2017).

The mechanism of azole antifungal resistance in candidiasis infection has several mechanisms, and some studies have been extensively studied such as *C. albicans*. DNA mutation in the ERG11 gene cause resistance mechanisms, and amino acid substitutions cause decreased fluconazole susceptibility (Marichal et al. 1999). Xiang reported that nine site directed mutations of ERG11 in 23 *C. albicans* isolates generated stronger fluconazole resistance, where the five amino acid substitutions produced may be located close to the active site of Erg11p (Xiang et al. 2013). Another fluconazole resistance mechanism is increased ERG11 expression which could induce mutations of genes involved in the zin cluster transcriptional regulator Upc2p (Whaley et al. 2017). Inactivation or deletion of the ERG3 gene encoding a sterol 15,6 desaturase, an enzyme involved in ergosterol biosynthesis, could be an alternative mechanism although there are few reports on it. In this mechanism, inactivation or inactivation in the absence of Ergp can prevent the synthesis of the toxic sterol 14α-methylergosta-8,24(28)-dien-3β,6α-diol (Morion et al. 2012; Whaley et al. 2017).

Although fluconazole is effective as an antifungal, in some cases *C.krusei* is resistant to fluconazole; however, this is not completely or clearly defined. It has been reported that some mechanisms involving Erg11p reduce azole affinity for Erg11p (Guinea et al. 2006; Lamping et al. 2009). Erg11p catalyzes the C14-demethylation of lanosterol which is critical for ergosterol biosynthesis (www.uniprot.org). Alteration of the cell membrane can affect membrane fluidity causing intracellular azole accumulation, which is also implicated in azole resistance (Kolaczkowska and Kolaczkowski 2016). In another report, overexpression of Erg11p and Abc2p, an efflux pump, might play an essential role in itraconazole resistance (He et al. 2015), but its detailed mechanism remains to be investigated.

5. Conclusions

M. citrifolia fruit extract has phytochemical and chemical compounds. It was found to be abundant in eucalyptol confirmed, as confirmed by its occurrence in two fractions. Furthermore, *M. citrifolia* fruit extract can inhibit the growth of *C. albicans* and *C. krusei*. The antifungal activity of this fruit can extended to the pharmaceutical and medical fields. *M. citrifolia* fruit extract has the potential as a natural agent to alleviate candidiasis vaginalis attacking the reproductive system of women.

Authors' Contributions: SUSILAWATI, S.: conception and design, acquisition of data, analysis and interpretation of data, and drafting the article; ANWAR, C.: critical review of important intellectual content; SALEH, M.I.: critical review of important intellectual content; SALNI: critical review of important intellectual content; HERMANSYAH, H.: analysis and interpretation of data; OKTIARNI, D.: acquisition of data and analysis and interpretation of data. All authors have read and approved the final version of the manuscript.

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