Original Article

Antifungal activities of *Medinilla speciosa* Blume fruit extracts against *Candida albicans* and *Trichophyton rubrum*

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ABSTRACT

Some antifungal drugs that are used to treat candidiasis and dermatomycosis can change the life cycle and growth patterns of fungi, leading to resistance. To avoid this, alternative medicines such as medicinal plants are needed. *Medinilla speciosa* Blume is a plant originating from Mount Muria, Kudus, Central Java, Indonesia, which has been used by the community to treat diarrhea, inflammation, and bacterial infections, but there is no information about its antifungal activity. This study aimed to determine the antifungal activity of *M. speciosa* Blume fruit extracts against *Candida albicans* ATCC 10231 and *Trichophyton rubrum* ATCC 28188. This research was performed by plant determination and sample preparation, sample extraction by gradually maceration, phytochemicals screening, TLC profile assay, antifungal activity test, Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) determination, and comparative antifungal activity study against Ketoconazole. The results showed that all of the extracts indicated no antifungal activity against *C. albicans* ATCC 10231, but methanol extract showed the strongest activity against *T. rubrum* ATCC 28188, followed by ethyl acetate and n-hexane extracts. MIC and MFC of methanol extract against *T. rubrum* ATCC 28188 were 391 and 781 ppm, respectively. The comparative antifungal activity value of methanol extract against Ketoconazole was 4621.68: 1. With regard to the results of phytochemical screening and TLC profile, the antifungal activity of methanol extract may be due to the presence of alkaloids, polyphenols, tannins, flavonoids, quinones, and saponins.

Keywords: *Medinilla speciosa* Blume, *Candida albicans*, *Trichophyton rubrum,* Ketoconazole

Introduction

One of the health problems in Indonesia is fungal infections, such as candidiasis and dermatomycosis [1-4]. During the past years, fungal infections have been increased due to the risen immunocompromised population (e.g., patients with AIDS or cancer, and organ transplant recipients). The prevalence of these diseases has been increased since the 1980s [5].

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Candidiasis is caused by *Candida albicans (C. albicans)*, a normal flora in the human body which in certain conditions becomes pathogenic and causes various diseases such as thrush, vulvanginitis, and even dangerous systemic candidiasis diseases. Generally, candidiasis treatment uses topical therapies, while systemic treatment is given to patients who experience resistance or show less effective response to topical therapies. Some antifungal drugs that are used to treat candidiasis include the azole group, nystatin, and amphotericin B [6]. Approximately, 69.5% of dermatomycosis in humans is caused by *Trichophyton rubrum (T. rubrum)*. This fungus usually infects the tissues containing keratin, such as nails, hair, and stratum corneum in epidermidis layer, which can expand into lesions [7]. Some antifungal drugs used to treat dermatomycosis are amphotericin B, azole group, alyl amine group, and griseofulvin [8, 9].

Most antifungal drugs can change the life cycle of fungi and growth patterns of fungi, leading to resistance in certain pathogenic fungi [10]. Therefore, the discovery of novel antifungal agents becomes a necessity, to widen the spectrum of antifungal activity against resistant fungi such as *C. albicans* and *T. rubrum*. The effort in exploring antifungal agents could be from conventional antifungal drugs, as well as from medicinal plants. Antifungal properties of some medicinal plants have been reported based on folklore data, while others are from studies on the inhibitory activity of the plants against some pathogenic fungi.

Medinilla speciosa (*M. speciosa*) Blume (Melastomaceae), is a plant originating from Mount Muria, Kudus, Central Java, Indonesia, which has been used by the community to treat diarrhea, inflammation, and bacterial infections [11]. Methanol, ethanol and ethyl acetate extracts of this fruit showed antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aureginosa*, *Bacillus subtilis, Methicillin-Resistant S. aureus* (MRSA), and *ESBL E. coli* [12]. *M. speciosa* Blume fruit contains various secondary metabolites, including terpenes, tannins, flavonoids, saponins, and glycosides. Antifungal activity study from *Melastoma malabathricum,* in the same family with *M. speciosa,* revealed that the extract inhibited the growth of *Candida krusei* [13]. Hence, the study on the antifungal activity of *M. speciosa* Blume fruit has not been reported yet. Therefore, this present study was conducted to determine the antifungal activity of *M. speciosa* fruit extract against *C. albicans* ATCC 10231 and *T. rubrum* ATCC 28188. It is expected that the data can be used as evidence of the potential of *M. speciosa* Blume fruit to be used as an alternative antifungal agent.

Materials and Methods

Materials

M. speciosa Blume fruit was collected from Mount Muria, Kudus, Central Java, Indonesia. *C. albicans* ATCC 10231 and *T. rubrum* ATCC 28188 were obtained from Hasan Sadikin Hospital, Bandung, West Java, Indonesia. The culture media were Potato Dextrose Agar/PDA (Oxoid) and Potato Dextrose Broth/PDB (Oxoid). Ketoconazole, as a reference antibiotic, was obtained from PT. Dexa Medica Indonesia.

The other materials used in this study were *n*-hexane (Merck), ethyl acetate (Merck), methanol (Merck), ammonia (Merck), chloroform (Merck), hydrochloric acid (Merck), Mayer reagent, Dragendorf reagent, Liebermann-Burchard reagent, magnesium powder (Bratachem), amyl alcohol (Merck), potassium hydroxide (Merck), iron (III) chloride (Merck), gelatin (Bratachem), ether (Merck), vanillin (Merck), dimethyl sulfoxide/DMSO (Merck), 96% ethanol (Bratachem), normal saline solution (Otsu-NS), 0.5 McFarland standard solution, TLC Silica Gel 60 F245 (Merck), paper discs 6 mm (Whatman), filter paper No. 1 (Whatman), and distilled water.

Plant determination and sample preparation

Leaves, fruits, stems, and roots of this plant were determined at Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang, West Java, Indonesia. The healthy fresh fruit was sorted, then washed with running tap water and distilled water to remove any adsorbed contaminant from the fruit surface. The cleaned sample was dried at room temperature and ground to a coarse powder.

Sample extraction

The sample was extracted by a gradual maceration using three types of solvents of polar (methanol), semipolar (ethyl acetate), and nonpolar (*n*-hexane) solvents. The dried fruit powder (182 g) was macerated with 1 L of *n*-hexane at room temperature for 24 h. Next, the sample was filtered using filter paper No.1. The extraction process was repeated several times until the last drop of the extract was colorless. The residual sample was dried. The remaining sample was subjected to extraction by maceration using methanol and ethyl acetate as solvents. The solvents were removed using a rotary evaporator at 45°C, then concentrated in a 60°C water bath until the solid mass of extracts was obtained. The extracts were observed organoleptically and continued by yields determination.

Phytochemicals screening

Phytochemical compounds of *M. speciosa* Blume fruit extracts were screened using standard procedures as described by Fansworth (1966) in the former publication [14].

Thin layer chromatography (TLC) profile assay

TLC profile assay of *n*-hexane, ethyl acetate, and methanol extract from *M. speciosa* Blume was carried by using the stationary phase of TLC Silica Gel 60 F245, while the mobile phase was determined from the optimization process. The plate was observed under visible light, UV light 254, and 366 nm. Rf value was determined for each spot.

Antifungal activity test

Antifungal activity of *M. speciosa* Blume fruit extracts was tested against *C. albicans* ATCC 10231 and *T. rubrum* ATCC 28188 using paper disc diffusion assay (Kirby-Bauer method) [15] with some modifications. The extracts stock solutions were prepared at a concentration of 50 % w/v in DMSO 2% v/v. The sample solutions were prepared by diluting the stock solution to give concentrations of 20, 30, 40, and 50 % w/v against *C. albicans* ATCC 10231 and 5, 10, 15, and 20% w/v against *T. rubrum* ATCC 28188.

About 20 mL of warm PDA was added to a sterilized petri dish and allowed to solidify at room temperature. Briefly, 50 µL of

fungus suspensions were objected to PDA using a sterile drigalski rod. The sterile paper disc containing 20 µL of the extract was placed on the agar. As a comparison, the positive (PDA inoculated with fungal suspension) and negative (PDA) controls were also prepared. All plates were incubated at 30°C for 1-2 days for *C. albicans* ATCC 10231 and 3-5 days for *T. rubrum* ATCC 28188. The inhibition zones were measured using calipers. All measurements were carried out in triplicate.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values

Determination of MIC and MFC values of the most potential extract of *M. speciosa* Blume fruit against *C. albicans* ATCC 10231 and/or *T. rubrum* ATCC 28188 were performed in 96-well microtiter plates, using two-fold broth microdilution methods in triplicates [16, 17] with some modifications according to procedures of Nejad [18]. The crude extract of *M. speciosa* Blume was dissolved in 2% v/v DMSO, at a concentration of 10 % w/v or 100,000 ppm served as a stock solution. For the assay, stock extract solutions were diluted twice with sterile distilled water to produce serial decreasing dilutions ranging from columns 3 to 12. The adjusted inoculum suspensions should be diluted in sterile normal saline solution, so that, after inoculation, each well contained approximately 5 × 10^2 - 2.5 × 10^3 CFU/mL for *C. albicans* ATCC 10231 and 0.4 × 10^4 - 5 × 10^4 CFU/mL) for *T. rubrum* ATCC 28188.

About 50 µL fungus suspension was inoculated against each well containing 50 µL of extract in the dilution series (and a positive control containing only broth) and were mixed thoroughly. Column 1 served as a negative control (PDB), while column 2 as a positive control was also used as a blank control to verify fungal growth. The microplates were then incubated at 30°C for 1-2 days for *C. albicans* ATCC 10231 and 3-5 days for *T. rubrum* ATCC 28188. The MIC value is the lowest concentration of extract to give complete inhibition recorded as visible growth.

The MFC was determined by introducing the solution from each well and cultured on PDA. The plates were incubated at 30°C for 1-2 days for *C. albicans* ATCC 10231 or 3-5 days for *T. rubrum* ATCC 28188. The lowest concentration producing no visible fungal growth after incubation was considered to be MFC value. Each fungus was tested in triplicate with 3 plates.

Comparative study of antifungal activity

The comparative study of antifungal activity was performed to determine the equivalence of antifungal activity of the most potential extract of *M. speciosa* Blume fruit compared with ketoconazole as a standard antifungal agent against *C. albicans* ATCC 10231 and/or *T. rubrum* ATCC 28188. The experiment was performed on the same plate using the same procedure as the antifungal activity test [15]. Ketoconazole was used as a standard due to its commonly used antifungal drug for the treatment of dermatomycoses [8].

The extract and Ketoconazole were dissolved and diluted to give various concentrations of samples. The log concentration values as x-axis were plotted against the inhibition diameter (mm) on the y-axis. The linear regression as a mathematical model was obtained and used to calculate comparative antifungal activity value. By comparing the concentration of *M. speciosa* Blume fruit extracts with the concentration of Ketoconazole producing the same inhibitory diameter, the comparison number can be evaluated.

Results and Discussion

Plant determination and sample preparation

The plant determination results showed that the plant is identic with *Medinilla speciosa* Blume from Melastomataceae family, Myrtales order, Magnoliopsida class, and Traceophyta division.

Sample extraction

Extraction was performed by maceration to avoid degradation of the thermolabile compound due to the heating process during extraction. The percentage yields of n-hexane, ethyl acetate, and methanol extracts were 3.34, 1.47, and 8.79% w/w, respectively. Characteristics of the extracts were thick, sticky, distinctive smelling with specific colour (light green for *n*-hexane, dark green for ethyl acetate and red brown for methanol extract).

It can be concluded that methanol extract gave the highest yield, followed by *n*-hexane and ethyl acetate extract. The results revealed that the compounds extracted from *M. speciosa* Blume fruit were dissolved more in the polar solvent than non-polar or semi-polar solvents.

Phytochemicals screening

The phytochemicals contained in *M. speciosa* Blume fruit extracts are presented in **Table 1**.

Table 1. Phytochemicals in *M. speciosa* Blume Fruit Extracts

Phytochemicals	*n*-hexane Extract	Ethyl Acetate Extract	Methanol Extract
Alkaloids	–	+	+
Polyphenols	+	+	+
Tannins	-	+	+
Flavonoids	-	+	+
Monoterpenes & Sesquiterpenes	–	+	–
Steroids & Triterpenes	+	–	–
Quinones	+	–	+
Saponins	-	+	+

+ : Presence, - : Absence

Considering **Table 1**, it can be seen that the *n*-hexane extract of *M. speciosa* Blume fruit contains polyphenols, steroids,

triterpenes, and quinones. Non-polar compounds, such as fat, steroids, coumarin, and some terpenes dissolve in *n*-hexane [19]. Furthermore, Ethyl acetate extract contains saponins, sesquiterpenes, monoterpenes, flavonoids, tannins, polyphenols, and alkaloids, while methanol extract contains saponins, quinones, flavonoids, tannins, polyphenols, and alkaloids. The compounds contained in the three *M. speciosa* Blume fruit extracts are polyphenols. Polyphenols, tannins, flavonoids, and saponins compounds can generally be dissolved by polar or semi-polar solvents, such as methanol and ethyl acetate [19]. Flavonoid compounds have polyhydroxy groups, so these compounds are more partitioned in semi-polar and polar solvents. Flavonoids and phenolic acids have been the object of various investigations concerning their antioxidant and antimicrobial activities.

TLC profile assay

Based on the optimization results, the mobile phase was ethyl acetate:*n*-hexane (3:7) for *n*-hexane and ethyl acetate extracts, while for methanolic extract was n-hexane: ethyl acetate (1:9). The result of the TLC profile assay of *M. speciosa* fruit extracts is shown in **Table 2**.

Table 2. TLC Profile of *M. speciosa* Fruit Extracts

Extract	Spot No.	Rf	Spot color under		
			Visible Light	UV 254 nm	UV 366 nm
n-hexane	1	0.21	brown	yellow	blue
	2	0.33	brown	yellow	blue
	3	0.48	green	yellow	blue
	4	0.61	brown	yellow	red
	5	0.73	green	yellow-greenish	red
	6	0.86	green	-	grey-brownish
	7	1.00	brown	green	brown
Ethyl acetate	1	0.16	-	blue	yellow-brownish
	2	0.31	brown	yellow	blue
	3	0.36	-	blue	brown
	4	0.43	-	blue	brown
	5	0.56	brown	yellow	red
	6	0.70	brown	yellow	red
	7	0.73	brown	yellow	red
	8	0.81	brown	green	grey
	9	0.85	-	-	grey
	10	0.88	brown	green	grey
	11	0.93	-	-	grey
Methanol	1	0.18	-	brown	-
	2	0.31	-	brown	-
	3	0.40	-	brown	blue
	4	0.51	-	brown	blue
	5	0.75	green	brown	green
	6	0.83	-	-	yellow
	7	0.93	-	-	brown
	8	0.96	green	brown	blue
	9	1.00	-	brown	grey

Concerning **Table 2**, it can be seen that the methanol and ethyl acetate extracts gave more spots than that from n-hexane extract. There are one or two yellow-greenish spots under visible light and blue fluorescence under UV light 366 nm indicating the presence of flavonoid compounds [20].

Antifungal activity test

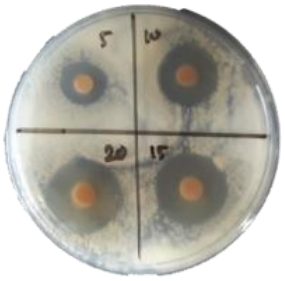
Antifungal activity of *M. speciosa* Blume fruit extracts against *C. albicans* ATCC 10231 and *T. rubrum* ATCC 28188 are listed in **Table 3** and **Figure 1**.

Table 3. Antifungal Activity of *M. speciosa* Extracts

Extract	Concentration (% w/v)	Inhibition Diameter of *C. albicans* ATCC 10231 (mm)	Concentration (% w/v)	Inhibition Diameter of *T. rubrum* ATCC 28188 (mm)
n-hexane	20	-	5	-
	30	-	10	-
	40	-	15	-
	50	-	20	-
Ethyl Acetate	20	-	5	7.40
	30	-	10	8.10
	40	-	15	12.30
	50	-	20	13.60
Methanol	20	-	5	14.10
	30	-	10	18.10
	40	-	15	20.70
	50	-	20	22.22

Paper Discs Diameter = 6 mm

Table 3 shows that neither *n*-hexane, ethyl acetate nor methanol extracts of *M. speciosa* Blume fruit extracts showed antifungal activity against *C. albicans* ATCC 10231. In contrast, antifungal activity against *T. rubrum* ATCC 28188 revealed that methanol extract of *M. speciosa* showed the strongest activity, followed by ethyl acetate and n-hexane extract with zero activity. The sensitivity of *C* increased gradually with the increased concentration of methanol and ethyl acetate extract.



(a)

(b)

Figure 1. Inhibition zone of a) methanol, b) ethyl acetate from *M. speciosa* Blume fruit extracts against *T. rubrum* ATCC 28188

Furthermore, the results of the phytochemical investigation showed that ethyl acetate extract contains saponins, sesquiterpenes, monoterpenes, flavonoids, polyphenols, tannins, and alkaloids, while methanol extract contains saponins, quinones, flavonoids, tannins, polyphenols, and alkaloids. The presence of saponins, flavonoids, tannins, and alkaloids either in methanol or ethyl acetate extract was hypothesized to give antifungal activity. Hence, this statement needs to be proven by further study. Moreover, the presence of flavonoids and alkaloids showed antimicrobial activities [21-26]. Another important phytochemical compound in ethyl acetate extract to triterpenes is also reported to possess numerous antimicrobial activities [27]. Natural triterpenoids are present in herbal medicines and some of them give evidence concerning the antifungal activity of natural sources [18, 27]. Several publications have documented the antifungal activities of essential oil and plant extracts [28, 29].

Determination of MIC and MFC values

Further study on determination of MIC and MFC values were conducted to methanol extract of *M. speciosa* Blume which shows the most potent antifungal activity. The MIC study was conducted against *T. rubrum* ATCC 28188. Concentrations of the extract, from third column to twelfth column were 50,000, 25,000, 12,500, 6,250, 3,125, 1,563, 781, 391, 196 and 98 ppm. The results of the determination of MIC and MFC value of methanol extract are shown in **Table 4**.

Table 4. Determination of MIC and MFC Value of Methanol Extract from *M. speciosa* Blume Fruit against *T. rubrum* ATCC 28188

Extract Concentration (ppm)	Fungal Growth in	
	PDB	PDA
50000	-	-
25000	-	-
12500	-	-
6250	-	-
3125	-	-
1563	-	-
781	-	-
391	-	+
196	+	+
98	+	+

+ : Presence, -: Absence

Considering **Table 4**, we found out that the MIC and MFC values of methanol extract from *M. speciosa* Blume fruit against *T. rubrum* ATCC 28188 are 391 ppm and 781 ppm, respectively. MFC value was elaborated as the lowest concentration which could inhibit the growth of *T. rubrum* ATCC 28188, which is shown by transparent PDA media where no fungal growth can be observed. Concerning the results of phytochemical screening, the antifungal activity of methanol extract may be due to the presence of alkaloids, polyphenols, tannins, flavonoids, quinones, and saponins. The finding was in line with the former study which stated that Alkaloids, terpenes (monoterpenes, diterpenes, triterpenes, sesquiterpenes, & sterols), polyphenols, flavonoids, quinones, tannins, and saponins contained in plants generally have antimicrobial activity [19]. Alkaloids compounds can interfere with the formation of peptidoglycan components in fungal cells, so that cell walls are not fully formed and cause cell death. Flavonoid compounds denature proteins that increase cell permeability, thereby damaging fungal cells and ultimately cell death. Tannins and polyphenols compounds can inhibit the synthesis of chitin as a major component in the cell wall of fungi. Saponins can reduce the surface tension of the sterol membrane which plays a role in the synthesis of the fungus cell wall. Quinones can interfere with cell wall permeability so that the occurrence of leakage in cellular substance is important in the growth of fungal cells [30].

Comparative study of antifungal activity

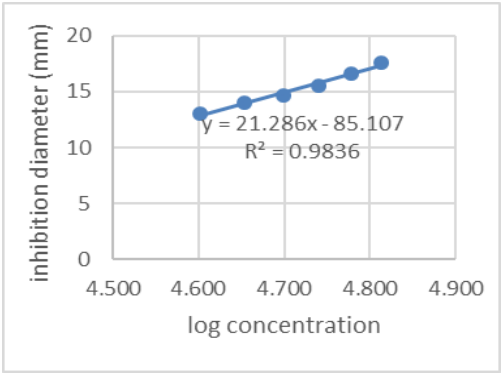
A comparative study of antifungal activity from methanol extract was performed at different concentrations of extract (40000, 45000, 50000, 55000, 60000, and 65000 ppm), and ketoconazole (5, 7.5, 10, 12.5, 15, & 17.5 ppm) as standard antifungal drug. The results from the antifungal activity study of methanol extract and ketoconazole against *T. rubrum* ATCC 28188 are shown in **Table 5**.

Table 5. The Antifungal Activity of Methanol Extract and Ketoconazole against *T. rubrum* ATCC 28188

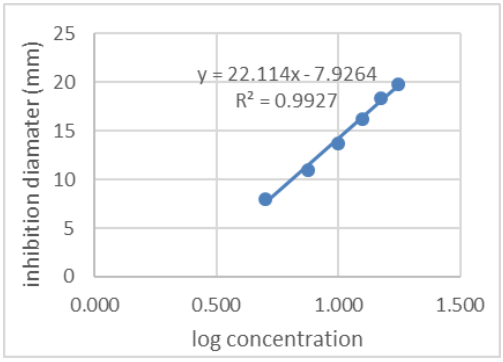
Subject	Concentration (ppm)	Inhibition Diameter (mm)*
Methanol Extract	40000	13.02
	45000	14.01
	50000	14.70
	55000	15.50
	60000	16.63
	65000	17.60
Ketoconazole	5	8.00
	7.5	11.02
	10	13.73
	12.5	16.23
	15	18.38
	17.5	19.76

*Paper Discs Diameter = 6 mm

The log of concentration on the x-axis was plotted against the inhibitory diameter on the y-axis. Either extract or ketoconazole provided linear regression equations as shown in **Figure 2**. In conclusion, the increase of the inhibition zone resulted from a particular concentration of either extract or ketoconazole can be predicted by a simple linear regression function.



(a)



(b)

Figure 2. Linear regression function for prediction of the effect of concentration a) methanol extract and b) Ketoconazole on inhibition zone of *T. rubrum* ATCC 28188

As an example of the calculation, from **Figure 2** it is known that the linear regression equation for ketoconazole was y = 22.114x – 7.9264 with $R^2 = 0.9927$. Applying this equation to calculate the inhibition zone, 10 ppm ketoconazole is predicted to give an inhibition diameter of approximately 14.1876 mm. Employing the linear regression equation of methanol extract to provide the same diameter of inhibition zone as that by ketoconazole, this diameter value is applied to the linear regression equation of methanol extract, y = 21.286x - 85.107 with $R^2 = 0.9836$. The result of the x value was 4.6648, therefore its antilog x was 46216.8136. It can be concluded that the comparative antifungal activity value of methanol extract from *M. speciosa* Blume fruit against ketoconazole was 46216.8136: 10. Accordingly, it can be concluded that to give the same inhibitory diameter with 1 ppm of ketoconazole, 4621.68136 ppm methanol extract from *M. speciosa* Blume fruit is needed.

The crude extracts concentrations play a key role in growth inhibition. Variation in results could be observed, which may be due to various factors such as environmental factors, the sample concentration, investigation methods, extraction methods, fraction used, the composition of plant content, and geographical origin and seasonal variation of the plants [29]. In

contrast, inhibition given by chemical drugs mostly will reveal reproducible results due to the highest impurity and reliability.

Conclusion

The methanol extract of *M. speciosa* Blume fruit showed promising antifungal activity compared to ethyl acetate and *n*-hexane, due to the more biologically active presence in the extract. This result revealed that the extract can be a candidate for alternative antifungals to treat dermatomycosis. The findings of the current study were in agreement with the traditional use of the plant in the treatment of microbial infections. Further research is needed to isolate active antifungal agents in a pure form for further pharmaceutical applications as an alternative for current antifungal treatments.

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Ethics statement: None

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