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Antibacterial potentials of *Rothmannia octomera* (*Rubiaceae*) root extracts on a few bacterial etiologies of respiratory tract infections

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Abstract

Rothmannia octomera is a plant of the Rubiaceae family used in traditional medicine to treat bronchopulmonary infections. To contribute to the valorization initiatives of this plant, we undertook a study of its root phytochemistry. We assessed its antibacterial potential against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, which are considered bacterial etiologies of respiratory infections. The phytochemical screening of the hydroethanolic (30:70 v/v) and CH_2Cl_2/MeOH (50:50 v/v) extracts of *Rothmannia octomera*, obtained by maceration, was carried out using colorimetric and precipitation tests for several groups of secondary metabolites. For the antibacterial potential of the extracts, minimal inhibitory and bactericidal concentrations (MICs and MBCs) were determined by macrodilution in a liquid medium. Results revealed the presence of phenols, tannins, flavonoids, sterols, anthraquinones, and saponins in both extracts. MIC values for the hydroethanolic extract ranged from 12.5 to 200 mg/mL, while MBC values varied from 50 to 400 mg/mL. For the CH_2Cl_2/MeOH extract, the MIC values recorded ranged from 50 to 200 mg/mL, and the related BMCs ranged from 100 to 200 mg/mL. The BMCs/MICs ratios revealed a bactericidal effect of the hydroethanolic extract on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, as well as a bacteriostatic potential on *Staphylococcus aureus*. With the CH_2Cl_2/MeOH extract, bactericidal action was observed against all the bacteria tested. This study justifies, for the first time, at least in part, the use of *Rothmannia octomera* in traditional medicine. Indeed, investigations on the safety of this plant and dosages required for its optimal use in disease control are necessary.

Keywords: *Rothmannia octomera*; *Pseudomonas aeruginosa*; *Klebsiella pneumonia*; *Staphylococcus aureus*; Antibacterial; Phytochemicals.

1. Introduction

Respiratory tract infections (RTIs) encompass a spectrum of disorders affecting both the upper and lower respiratory tracts. These infections are caused by a diverse range of pathogenic microorganisms, including viruses, bacteria, and fungi, and are associated with varied clinical manifestations [1]. These pathogens are significant contributors to both community-acquired and

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hospital-acquired respiratory infections, accounting for nearly 4 million deaths annually across all age groups globally [2]. The burden of RTIs is particularly pronounced in sub-Saharan Africa, where they are responsible for 25% of mortality among children under five years of age [3].

The management of infectious diseases through antibiotics has been hindered by their high cost and limited accessibility, particularly in resource-constrained settings. This has led to the widespread and often irrational use of antibiotics in empirical and probabilistic therapies in human medicine, as well as in crop production and animal husbandry. Such practices are recognized as key drivers in the emergence and dissemination of antimicrobial resistance phenotypes and genotypes among bacterial pathogens [4]. The escalating prevalence of antimicrobial resistance has further complicated therapeutic strategies, underscoring the urgent need for the development of alternative treatment options.

In light of increasing concerns regarding the cost, toxicity, and the emergence of tolerant bacterial strains, the use of antibacterial agents derived from medicinal plants has become a viable treatment strategy for controlling bacterial infections. These plant-based agents are characterized by their readily available chemical constituents, which are often more affordable and capable of acting through novel mechanisms on bacteria with adaptable genotype expressions [4, 5]. This emerging research direction is strongly endorsed by the World Health Organization (WHO), particularly for the approximately 80% of the African population that depends on medicinal plants as a primary resource within the healthcare system [5-7].

The genus *Rothmannia octomera*, a member of the Rubiaceae family, is a plant species that has been traditionally used in folk medicine for its purported medicinal properties. It encompasses approximately 40 species that grow in tropical Africa, the Seychelles, Asia, and America. Known in Cameroon as "oyebe'e" (in the Bulu language), *Rothmannia octomera* is a shrub that grows 1-7 meters high [8] and is often used to treat bronchopulmonary infections [9]. Similarly, the leaves and bark of *Rothmannia octomera* are used in traditional medicine to treat urinary tract infections [10]. While previous studies [11-13] have highlighted the pharmacological potential of various plant species, the antibacterial properties of *Rothmannia octomera* remain underexplored, particularly with respect to its root extracts. Given the increasing demand for new antimicrobial agents, this study aims to investigate the antibacterial potential of *Rothmannia octomera* root extracts against a few bacterial etiologies of respiratory tract infections. By elucidating the efficacy of these extracts, this research seeks to contribute to the growing body of knowledge on plant-derived antimicrobials and their potential role in addressing the challenge of antibiotic resistance.

2. Material and methods

2.1. Plant material

The root sample of *Rothmannia octomera* was collected nearly 25 km South of Yaounde, at Mont Kala in the district of Mbankomo, Centre Region, Cameroon. The plant was identified taxonomically and preserved as a voucher specimen (number 43517/HNC) at the Cameroon National Herbarium for future use.

2.2. Subjected microorganisms

The bacteria subjected consisted of clinical isolates selected based on their involvement in respiratory tract infections. They included *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* that the Laboratory of Microbiology provided at the University Clinics des Montagnes, Université des Montagnes. The antibacterial potential was tested in the same laboratory.

2.3. Extracts preparation

The roots of *Rothmannia octomera* were cut into small pieces (≈ 1 cm) and dried for 12 days at 20°C in a sunlight-free environment, and were then grounded into a fine powder; after which, 5 liters of CH_2Cl_2/MeOH (50:50 v/v) were added into 900 g of the root powder and 2 liters of EtOH/H_2O (70:30 v/v) were added into 340 g of the root powder; the mixtures were kept in storage for 72 h to allow for complete mixing with periodic shaking and stirring. The resulting macerates were filtered through a Whatman® N° 2 paper and concentrated in a rotary evaporator (Heidolph®) at 40°C. The resulting extracts (hydroethanolic and Dichloromethane/methanol) were stored at 40°C for 72 hours to remove residual solvents.

2.4. Phytochemical screening of Rothmannia octomera root extracts

The phytochemical screening was carried out according to the method of Bruneton [14] for the two *Rothmannia octomera* root extracts (hydroethanolic and Dichloromethane/methanol).

2.4.1. Test for flavonoids: Shinoda test

A mass of 5 mg from each extract was dissolved in 1 mL of methanol in two separate test tubes. Into each of these tubes, 0.05 g of magnesium chips and 3 drops of concentrated hydrochloric acid were added for the preparation. The colors yellow or red observed indicate the presence of flavone, while a pink color indicates the presence of flavonones.

2.4.2. Test for phenols: Ferric Chloride Test

In two test tubes, 5 mg of each extract was dissolved separately in 1 mL of ethanol. Three drops of 10% $FeCl_3$ were added to each mixture. A blue-blackish or greenish color developing indicated the presence of phenols in the extracts.

2.4.3. Test for tannins: Stiasny Test (chloridic acid/ Formalin) at 30%

Three drops of Stiany's reagent were added to 5 mL of each extract in two separate test tubes. The mixture obtained is heated in a water bath at 90°C for 15 minutes. A brown precipitate was recorded, indicating the presence of catechic tannins in the extracts. The previous solution was filtered, and the filtrate was saturated with sodium acetate. Then, three drops of ferric chloride were added. The appearance of an intense blue-black color indicated the presence of Gallic tannins in the extracts.

2.4.4. Test for alkaloids: Mayer test

In two test tubes, 5 mg of each extract was dissolved separately in 1 mL of methanol. Thereafter, 1 mL of 1% H_2SO_4 was added to each tube. The mixtures were boiled in a water bath for 5 minutes. After cooling and filtration, five drops of Mayer's reagent were added to the filtrates. When a yellow-white precipitate was observed, it indicated the presence of alkaloids.

2.4.5. Test for saponins

In two test tubes, each containing five mL of distilled water, five mg of each extract was added separately and heated for 5 minutes. After cooling, the preparation in each tube was shaken vertically for 15 seconds and then left to stand. The presence of one-centimeter-high persistent foam indicated the presence of saponins.

2.4.6. Test for triterpenes and sterols: Liebermann-Burchard test

A mass of 5 mg of each extract was dissolved separately into two test tubes, each containing 1 mL of methanol. To these mixtures, 0.2 mL of chloroform, glacial acetic anhydride, and concentrated H_2SO_4 were added. A violet or greenish color recorded indicated the presence of triterpenes and sterols in the extracts, respectively.

2.4.7. Test for anthocyanins

In two test tubes, 5 mg of each extract was dissolved separately in 1 mL of methanol. Five drops of concentrated hydrochloric acid were added to each test tube in the preparation. The development of an orange color in the extracts was evidence of the presence of anthocyanins.

2.4.8. Test for coumarins

In two test tubes, 3 mg of each extract was dissolved separately in 1 mL of methanol. Then 0.5 mL of 25% ammonia was added to each of the test tubes. The resulting preparation was homogenized and observed under UV light (at wavelength λ = 365 nm). An intense blue fluorescence was evidence of the presence of coumarins.

2.4.9. Test for anthraquinones

In two test tubes, 5 mg of each extract was dissolved separately in 1 mL of ether chloroform (1:1). The solutions were treated with 4 mL of 10% sodium hydroxide; a resulting reddish color indicates the presence of anthraquinones.

2.5. Determination of total phenolic compounds and flavonoid contents

Total polyphenol compounds were determined by the modified Folin-Ciocalteu method [15], with tannic acid as the standard. 500 µL of Folin-Ciocalteu reagent and 2 mL of 20% (m/v) Na_2CO_3 were added to 100 µL of each extract. The mixtures were stirred and incubated at room temperature for 30 minutes in a dark environment. The

absorbance was then read at 760 nm using a UV spectrophotometer (PerkinElmer), and the results were expressed in mg tannic acid equivalent/g of dry weight specimen, with reference to the tannic acid calibration curve.

Total flavonoids were estimated according to Mohammadhosseini *et al.* [16]. A mixture of 100 µL of 10% (w/v) $AlCl_3$, 100 µL of 1 M sodium acetate, 1.5 mL of 95% methanol, and 2.8 mL of distilled water was added separately to 500 µL of each extract. The preparations were stirred and incubated at room temperature for 30 min in a dark environment. The blank was prepared by replacing the extract with 95% methanol. The absorbance was subsequently measured at 415 nm using a UV spectrophotometer (PerkinElmer), and the results were expressed as mg quercetin equivalent/g of dry weight specimen, with reference to the quercetin calibration curve.

2.6. In vitro evaluation of the antibacterial potential of hydroethanolic and dichloromethane/methanol extracts of the roots of Rothmannia octomera

2.6.1. Test of extract antibacterial activity

An activity test based on the antibiogram principle was carried out. Mueller-Hinton agar (MHA) in 90-mm Petri dishes was brought close to the flame of a Bunsen burner and inoculated with the bacterial inoculum (0.5 McFarland) in tight streaks using a sterile swab. Sterile six mm-diameter Whatman disks were then gently placed on the surface of the agar with a bacterial lawn. Thereafter, the disks were impregnated with 15 µL of the extract previously solubilized in distilled water. After 10 minutes on the workbench, the preparations in Petri dishes were incubated for 24 hours at 37°C. Extracts at 100 mg/mL and 200 mg/mL concentrations were tested, and the extract was considered active against the subject bacterial isolate when a zone of inhibition was observed upon completion of incubation (this was a prerequisite for further investigations through MICs and MBCs).

2.6.2. Investigation through the Minimum Inhibitory Concentrations (MICs)

The minimum inhibitory concentration (MIC) refers to the lowest concentration of an antimicrobial that prevents cell division under optimal growth conditions. The MICs were determined according to the following protocol: thirteen test tubes were used per bacterial isolate, labeled 1 through 10 for tests and 3 controls namely, reagent control (RC), negative control (NC) and positive control (PC). Initially, 1000 µL of Mueller Hinton broth (MHB) was dispensed into the 13 tubes. Then, 1000 µL of stock extract solution was added to the first tube of the series from which a serial dilution (two-fold) was conducted to concentrations ranging from 400mg through 0.781mg/mL for the hydroethanolic extract and 200 through 0.391mg/mL for the CH_2Cl_2/MeOH extract. In each preparation in test tubes (including the positive control tube), 15 µL of bacterial inoculum (0.5 McFarland) was added. All the test and control preparations were gently homogenized with a vortex mixer prior to incubation for 24 hours at 37°C. Upon completion of incubation, the MIC was primarily assessed visually in the test tubes based on apparent turbidity with reference to the control tubes; then after centrifugation at 5,000 rpm for 5 minutes, for eventual bacterial deposits that further testified bacterial growth, all the tests were carried out in triplicate.

2.6.3. Investigating through the Minimal Bactericidal Concentrations (MBCs)

The minimum bactericidal concentration (MBC) refers to the lowest concentration that kills more than 99.9% of the initial bacterial inoculum (i.e., less than 0.11% survivors) under optimal growth conditions. The BMCs were determined from the MICs' tubes. In each tube of the dilution range where turbidity was not observed, and in the control tubes, the re-homogenized preparation was streaked onto Mueller-Hinton agar (MHA) in Petri dishes. The resulting preparations were incubated for 18 hours at 37°C. Upon completion of incubation, the BMC was recorded from the first concentration in which no bacterial growth was observed on MHA**.** All tests were carried out in triplicate, as was the case for the MICs.

The BMCs/MICs ratios were calculated to determine the bactericidal or bacteriostatic potential of *Rothmannia octomera* extracts on the bacteria subjected (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*). An extract is regarded as bactericidal if this ratio is smaller than or equal to 4 (BMC/MIC ≤ 4), as bacteriostatic if this value is larger

than 4 (BMC/MIC > 4), or absolutely bactericidal if the MIC is equal to the MBC (BMC/MIC = 1).

3. Results and Discussion

The phytochemical screening revealed the presence of phenols, tannins, flavonoids, sterols, saponins, and anthraquinones in both extracts under study, while anthocyanins were absent. However, coumarins were present in the hydroethanolic extract and not in the dichloromethane/methanol extract. Alkaloids and triterpenes were detected only in the dichloromethane/methanol extract. Further details on the composition of these extracts are summarized and displayed in **Table 1**.

Table 1 indicates that the difference recorded in the secondary metabolite groups is primarily likely associated with the extraction solvent used. In fact, during the process of maceration, the hydroethanolic mixture (30:70/v:v) acts more efficiently on polar compounds, whereas the CH_2Cl_2/MeOH solvent (50:50/v:v) acts and extracts compounds of variable polarities. The secondary metabolites detected in these extracts are widely recognized for their anti-inflammatory, analgesic, vasodilatory, antioxidant and antimicrobial properties [13, 17-19].

Table 1. Phytochemical composition of dichloromethane/methanol and hydroethanolic extracts of plant roots

Class of secondary metabolites	CH_2Cl_2/MeOH Extract	EtOH/H_2O Extract
Flavonoids	+	+
Phenols	+	+
Tannins	+	+
Anthocyanins	-	-
Triterpenes	+	-
Sterols	+	+
Saponins	+	+
Anthraquinones	+	+
Alkaloids	+	-
Coumarins	-	+

- No staining; + staining or precipitation; (Staining or precipitation indicates the presence of secondary metabolites).

In the present study, the flavonoids assay revealed that their content was higher in hydroethanolic extracts (**Figure 1**). The relative affinity of this class of compounds for the extraction solvent systems used could justify this result. However, the polyphenol contents of the hydroethanolic extract of *Rothmannia octomera* roots were higher than those of the CH_2Cl_2/MeOH. The difference in polyphenol content in the extracts under study could be attributed to the presence of coumarins in this extract and the richness in flavonoids observed in the hydroethanolic extract. In addition, polyphenols have a high affinity for polar solvents and, therefore, are more soluble in hydroalcoholic solvents [20].

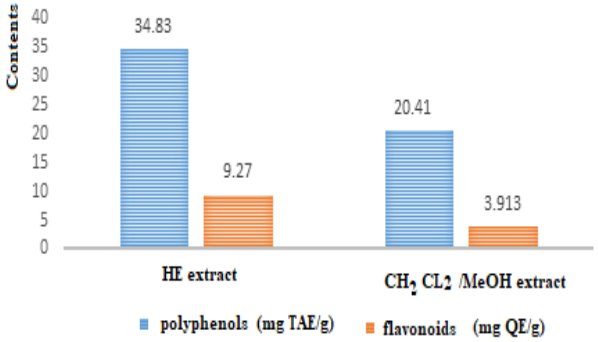

Figure 1. Polyphenol and flavonoid contents of *Rothmannia octomera* root extracts.
HE: Hydroethanolic Extract; CH_2CL_2/MeOH: Dichloromethane/methanol Extract; TAE: tannic acid equivalent; QE: quercetin equivalent.

Previous related findings [21-23] have been reported on other species belonging to the *Rothmannia* genus. An investigation on the ethanolic extracts of *Rothmannia talbotii*'s woods, as reported by Koagne *et al.* [21], isolated seven compounds that typically belonged to the iridoid and phytosterol classes. Similarly, Chaipukdee *et al.* [22] isolated eight compounds that belonged to the iridoid class, one from the phytosterol class and two phenolic compounds, with n-hexane and EtOAc extracts obtained from the bark and methanolic extract from the fruit of *Rothmannia wittii*. Together, these results indicate that plants belonging to the genus *Rothmannia* contain a high diversity of metabolic groups. This may explain their potential as liver protector, blood sugar level reducer, neuroprotector and cholesterol-reducer [23]; just as their spatial distribution.

The antibacterial activities of the hydroethanolic and dichloromethane/methanol extracts from the roots of *Rothmannia octomera* have been evaluated against

several bacteria known to cause respiratory tract infections. The inhibition zones observed around the disks indicated variations among different bacterial types, testifying to their antibacterial potential at concentrations of 100 and 200 mg/mL. Cowan *et al.* [13] observed that plant richness in certain metabolites provides antimicrobial properties, as they serve as protection against environmental stresses. This view is consistent with the findings reported in the present study, which partially justifies the spatial distribution introduced earlier. According to some authors [17-19, 24], anthraquinones inhibit bacterial growth by inactivating proteins, while tannins exert their antibacterial activity by denaturing enzymatic proteins and chelating certain ions involved in the vital features of some bacteria.

Table 2 presents MIC values of the hydroethanolic extracts as lower than those of the dichloromethane/methanol extracts for *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The differences in MIC values recorded may be attributed to the higher levels of polyphenols in the hydroethanolic extract. According to Mahmoudi *et al.* [20], hydroalcoholic solvents provide a more effective extraction of polyphenols. Polyphenols are endowed with a wide range of biological activities, including antibacterial potential through the inhibition of bacterial growth by increasing the permeability of the cell envelope, which results in cell lysis, disrupting the DNA supercoiling mechanism, or inhibiting certain important bacterial protein functions [13, 17, 18]. These differences in MIC values could also be due to other metabolites that were not investigated in the present study.

Based on the results from the qualitative screening of the extracts, the dichloromethane/methanol extract would theoretically be expected to have lower MIC values (more active) than the hydroethanolic extract, because the dichloromethane/methanol extract contains triterpenes and alkaloids that were not detected in the hydroethanolic extract. In fact, Triterpenes can infiltrate the lipid bilayer and cause rupture of bacterial membrane [18], while alkaloids inhibit bacterial growth by intercalating into the DNA [13], likely affecting the supercoiling process. These views are in line with the stochastic scenarios associated with the diversity of metabolites that is hardly (if ever) exhaustive. The antibacterial potential of an extract could therefore be associated with its qualitative and quantitative contents in active metabolites.

The methanolic extract of *Rothmannia longiflora* leaves was reported to be active against Gram-negative and Gram-positive bacteria, including those investigated in the present study [25]. This report presented MIC values of 100 mg/mL for *Staphylococcus aureus*, while the values for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were 200 mg/mL. These MIC values were therefore higher than those recorded on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the present study, indicating greater activities of the extracts of *Rothmannia octomera*. The lower MIC values recorded in the present study could be due to the presence of flavonoids found in the hydroethanolic extract of *Rothmannia octomera*, which is not detected in the methanolic extract of *Rothmannia longiflora* leaves according to Awosan *et al* [25] and Udia *et al*. [26], who worked on the antibacterial activity and on the phytochemistry of this plant, respectively. Following these postulates, a geographical impact on the plant composition could be considered, since previous authors [25, 26] cited in the literature harvested the plant in Nigeria, while *Rothmannia octomera* roots were harvested in Cameroon.

Table 2. MIC, MBC, and MBC/MIC ratios of the various plant extracts

Bacteria isolate	Hydroethanolic extract			Dichloromethane/methanol extract		
	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI
K. pneumoniae	200	400	2	200	200	1
P. aeruginosa	50	100	2	100	200	2
S. aureus	12,5	50	4	50	100	2

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

Generally, flavonoids inhibit bacterial growth by increasing the permeability of the cell plasma membrane [13, 18]. Because of this, the activity of a plant may therefore depend on the composition of its active metabolites within the part of the plant used or on the place where the raw material is harvested.

Klebsiella pneumoniae, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are known etiologies of opportunistic community infections that are often difficult to control due to their resistance to available drugs. The lowest MIC values for the two extracts were recorded on *Staphylococcus aureus*, indicating greater activity against this bacterium. This result falls in line with reports from Bishnu *et al.* [27], Cock [28], and Ouattara *et al.* [29], who reported that plant extracts are more active on Gram-positive bacteria. Gram-positive bacteria consist of a cell wall made of several layers of peptidoglycan (approximately 90% of the bacterial wall), which is highly permeable to antibacterial agents. In comparison, Gram-negative bacteria have a thinner cell wall (5-20% of the bacterial wall). They are overlayered by a bilayer of phospholipids, which drastically reduces the permeability of the cell envelope [30]. In the present investigation, the highest MICs and BMCs were observed in *Klebsiella pneumonia*, a member of the Gram-negative group of bacteria that develops capsules, which, theoretically, could act to further reduce bacterial cell envelope permeability. In fact, the developed capsules are generally known to play important roles in bacterial resistance to environmental stresses.

Regarding CMBs/CMIs ratios, the hydroethanolic extract was bactericidal against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and bacteriostatic against *Staphylococcus aureus*, while the dichloromethane/methanol extract was bactericidal against all the isolates tested. Altogether, these findings justify (at least partially) the use of *Rothmannia* roots in traditional medicine in the management of infectious diseases, particularly for respiratory tract infections. From a benefit-to-risk point of view, the hydroethanolic extract would be suitable for formulation in phytotherapy due to its reduced toxicity compared to the dichloromethane/methanol extract.

4. Conclusion

The present study provided evidence of the antibacterial potential of *Rothmannia octomera* root extracts against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. It also revealed that *Rothmannia octomera* possesses bioactive phytochemical compounds that could be used in phytomedicine, especially for the control of bacterial infections caused by both gram-positive and gram-negative bacteria. Thus, *Rothmannia octomera* appears to be a potential candidate for further evaluation with a view to integrating ethnomedicine into medical practices.

Conflict of interest

The authors declare to have no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors Contributions

R.V.T.S and P.R.F.K: Study design, conceptualization, and supervision. S.T.M and O.T.T: Data collection, software, graphics. S.T.M: Data collection and literature search. R.V.T.S and O.T.T: Data analysis and writing the original draft. R.V.T.S and P.R.F.K: review, and editing. V.T-N: Review, editing and supervision.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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