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SOLVENT-DEPENDENT ANTIBACTERIAL, PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITIES OF *Zingiber officinale* RHIZOME FROM KAURA LOCAL GOVERNMENT AREA OF SOUTHERN KADUNA, NIGERIA

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ABSTRACT

The increasing prevalence of antimicrobial resistance and oxidative stress–related disorders has stimulated interest in medicinal plants as sources of bioactive compounds. This study evaluated the antibacterial, phytochemical, and antioxidant activities of *Zingiber officinale* rhizome extracts obtained from Kaura Local Government Area of Southern Kaduna, Nigeria. Powdered rhizomes were extracted using n-hexane, ethyl acetate, and methanol by cold maceration. Antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* was determined using the agar well diffusion method, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were evaluated using broth dilution assays. Phytochemical screening and antioxidant activity were assessed using standard qualitative methods, total phenolic and flavonoid content determination, and the DPPH radical scavenging assay. The methanol extract exhibited the strongest antibacterial activity, producing inhibition zones of 13.00–18.00 mm at 50 mg/mL, with *S. aureus* showing the highest susceptibility (MIC = 12.5 mg/mL). Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, terpenoids, glycosides, and saponins. The methanol extract showed the highest total phenolic content (1907.67 mg GAE/100 g), whereas the ethyl acetate extract had the highest flavonoid content (207.33 mg QE/100 g). Antioxidant evaluation demonstrated concentration-dependent radical scavenging activity, with the methanol extract exhibiting the strongest activity (IC_{50} = 30.12 µg/mL). These results highlight the potential of *Z. officinale* from Southern Kaduna as a natural source of antibacterial and antioxidant compounds.

Keywords: *Zingiber officinale*, Antibacterial activity, Antioxidant activity, Phytochemicals, DPPH assay

INTRODUCTION

Medicinal plants have long served as vital sources of therapeutic agents, particularly in the management of infectious diseases and oxidative stress–related disorders (Ashraf *et al.*, 2024). Among these, *Zingiber officinale* Roscoe (ginger) is one of the most widely studied and utilized plants due to its rich profile of bioactive phytochemicals and diverse biological activities (Mao *et al.*, 2019). Traditionally consumed as a spice and ethnomedicinal herb, ginger has been reported to exhibit significant antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects, attributed largely to its phenolic acids, flavonoids, gingerols, shogaols, and other secondary metabolites (Ayustaningwarno *et al.*, 2024).

Recent investigations have underscored the antibacterial efficacy of ginger extracts against a broad range of pathogenic bacteria, including both Gram-positive and Gram-negative strains, highlighting its potential role in addressing rising antimicrobial resistance globally (Zouine *et al.*, 2024). Methanolic and aqueous extracts of *Zingiber officinale* have demonstrated noteworthy inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, and other clinically relevant bacteria, with significant zones of inhibition and favorable MIC values reported in vitro (Koparde *et al.*, 2024; Bulakarima *et al.*, 2025). Additionally, advanced phytochemical profiling coupled with computational studies has revealed a complex spectrum of bioactive compounds that contribute to these antimicrobial actions, further supporting the pharmacological potential of ginger preparations from diverse cultivation regions (Sulieman *et al.*, 2024).

Parallel to its antibacterial properties, ginger has been consistently recognized for its antioxidant capacity, with multiple studies confirming its ability to scavenge free radicals and reduce oxidative stress through mechanisms linked to its phenolic and flavonoid contents (Ballester *et al.*, 2023). Quantitative antioxidant assays such as DPPH and ABTS have recorded significant free radical scavenging activity for various ginger extracts, pointing to its utility in mitigating oxidative damage associated with chronic diseases (Bulakarima *et al.*, 2025). Furthermore, comparative analyses indicate that the antioxidant potential may vary depending on extraction solvents, geographical origin, and the specific phytochemical composition of the rhizome (Ivanović *et al.*, 2021).

Despite these advances, there remains a relative paucity of data on the biological properties of *Z. officinale* cultivated in specific agro-ecological zones such as the Southern Kaduna region of Nigeria, where unique environmental factors may influence the phytochemical profile and consequent bioactivities. Understanding the antibacterial, antioxidant, and phytochemical characteristics of locally sourced ginger is essential not only for validating traditional uses but also for exploring its potential development into standardized therapeutic agents. Therefore, the present study aims to provide a comprehensive evaluation of the antibacterial and antioxidant activities, alongside detailed phytochemical screening of *Z. officinale* rhizomes obtained from Kaura Local Government Area of Southern Kaduna, with the goal of contributing novel insights into the medicinal potential and value of this indigenous plant resource.

MATERIALS AND METHODS

Plant Samples Collection, Preparation and Extraction

The ginger (*Zingiber officinale*) rhizome was harvested in Kagoro, Kaura Local Government area in Kaduna State, Nigeria. The sample after collection was presented to the

Department of Biological Sciences, Kaduna State University for authentication by a botanist (Dr. Ibrahim Bello). The sample was thoroughly washed and rinsed with distilled water, sliced into smaller pieces, and then dried at room temperature. The dried pieces were pulverized into powder using pestle and mortar, then the ground material was passed through a weave filter with a 60 mm opening to produce a fine powder. The obtained powder was then utilized in the preparation of the extracts (n-hexane, ethyl acetate and methanol). The crude extracts were made according to the cold extraction technique; 30g of the dried powder was soaked for 48 h in 300 mL each of n-hexane, ethyl acetate and methanol. The mixtures were then filtered again using Whatman filter paper and dried in the vacuum using a rotary vacuum evaporator (Edo, 2024).

Collection and Reconfirmation of the Test Bacterial Isolates

The clinical isolates of *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* were collected from the Garkuwa Specialist Hospital, Kaduna State. The isolates were transported in a sterile condition to the Microbiology laboratory, Kaduna State University. The collected bacterial strains were subcultured on nutrient agar and nutrient broth to obtain fresh bacterial growth. The inoculated media plates were kept for a further 24 h at 37°C. Following 24 h incubation at 37°C, the isolates were confirmed using cultural, morphological and bacteria identification kits (such as Microgen® GN-ID, Microgen® Staphylococcus-ID and Microgen® Enterococcus-ID) following the manufacturer's instruction.

Preparation of Different Concentration of the *Zingiber officinale* Extracts

The stocks solutions of the *Z. officinale* extracts were prepared by aseptically weighing 1g of the extract and dissolving in 5ml of Dimethylsulfoxide (DMSO) to 200 mg/mL solution. Thereafter, the different working concentrations (50, 25, 12.5 and 6.25 mg/mL) were prepared from the stock solution. Ciprofloxacin was used at 5g/ml concentration as positive control against every bacterial strain.

Determination of Antibacterial Activity of *Zingiber officinale* Extracts

The determination of antibacterial activities of the *Z. officinale* extracts were carried out using agar well diffusion method demonstrated by Hazir and Keskin (2020). On the freshly prepared Mueller Hinton agar, a sterile cork borer was used to produce holes of 6mm diameter. The plates were then inoculated with standardized test bacteria inoculum (0.5 McFaland). Each of the prepared extracts concentrations (50, 25, 12.5 and 6.25 mg/mL) was placed into their respective holes while the control (Ciprofloxacin at 10 µg/ml) at the middle of the plate. The prepared plates were allowed to diffuse for some time and then incubated overnight at 37ºC. After incubation period, the diameter of inhibition zones was measured in millimeter using ruler (Hazir and Keskin, 2020).

Determination Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The lowest concentration of an antimicrobial substance required to prevent the visible growth of a microorganism is termed as the Minimum Inhibitory Concentration (MIC). This was evaluated using broth dilution assay, with clear endpoints indicating microbial inhibition. The extracts working concentrations (50, 25, 12.5 and 6.25 mg/mL) were introduced into different test tube containing Mueller Hilton Broth (MHB). The tubes were then inoculated with 0.1ml of standardized (0.5 McFaland) inoculum and incubated overnight at 37°C to observe turbidity (growth). The least concentration showed no visible sign of turbidity of the medium was regarded as the MIC. The MBC was evaluated by sub-culturing the contents of the test tubes onto sterile Mueller Hilton Agar (MHA) and incubated overnight at 37°C. The MBC value was taken as the lowest concentration that totally inhibited the test organisms (Mann and Markham, 2018).

Phytochemical Screening

The secondary metabolites (alkaloids, anthraquinonne, glycoside, saponins, tannins, terpenoids, total flavonoids and total phenol) were determined through phytochemical screenings as described by Nwosu (2022). The phytochemicals present in the extracts of *Z. officinale* were determined.

Antioxidant Study

Total Phenolic and Flavonoid Contents

Total phenolic content was analyzed using the Folin Ciocalteu colorimetric method and the results were expressed as mg gallic acid equivalent (GAE) per gram extract (Ahmed *et al.*, 2022). On the other hand, the total flavonoid content was determined using the aluminum chloride colorimetric method. The results expressed as mg quercetin equivalent (QE) per gram of extract. Each plant extract was prepared in triplicate (Sulastri *et al.*, 2018).

DPPH Assay

The DPPH radical scavenging activity of the *Z. officinale* extracts at varying concentrations (1.57–50 mg/mL) was measured *in vitro* using 2, 2′- diphenyl-1- picrylhydrazyl (DPPH) assay. The extract concentrations providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition (%) against extract concentration. Ascorbic acid at the same concentrations was used as the reference antioxidants (Alfuraydi *et al.*, 2024).

Statistical Analysis

The statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) software version 26. Means and standard deviations (SD) were used to summarise continuous data. DPPH scavenging activity at different concentrations and half of minimum inhibitory concentration (IC_{50}) was determined by linear regression analysis method. Each sample was analyzed individually in triplicates and the results are expressed as the mean value (n = 3) ± standard deviation.

RESULTS AND DISCUSSION

The antibacterial activity of *Zingiber officinale* extracts against the tested bacterial isolates is presented in Table 1. The results demonstrate a clear variation in antibacterial efficacy among the solvent extracts, with the methanol extract exhibiting the highest inhibitory activity. The inhibitory zones ranged from 13.00 ± 0.67 to 18.00 ± 0.00 mm at the highest concentration (50 mg/mL), with *Staphylococcus aureus* showing the greatest susceptibility (18.00 ± 0.00 mm), followed by *Enterococcus faecalis* (14.67 ± 0.44 mm), *Escherichia coli* (14.33 ± 0.44 mm), and *Pseudomonas aeruginosa* (13.00 ± 0.67 mm).

The antibacterial activity decreased with decreasing extract concentration, suggesting a concentration-dependent inhibitory effect of the ginger extracts. Such dose-dependent

antimicrobial responses are commonly reported for plant-derived bioactive compounds due to increased availability of active metabolites at higher concentrations (Yit *et al.,* 2024). Notably, the methanol extract demonstrated stronger antibacterial activity against Gram-positive bacteria (*S. aureus* and *E. faecalis*) compared with Gram-negative bacteria (*E. coli* and *P. aeruginosa*). This observation may be attributed to structural differences in bacterial cell envelopes. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides that acts as an effective permeability barrier to many plant-derived antimicrobial compounds, thereby reducing susceptibility (Nourbakhsh *et al.,* 2022). Similar patterns of antibacterial susceptibility have been reported in recent studies investigating ginger extracts against pathogenic bacteria (Raeisi *et al.,* 2025). The ethyl acetate extract exhibited moderate antibacterial activity only at the highest concentration tested, with inhibition zones ranging from 8.00 ± 0.00 to 11.33 ± 0.44 mm, whereas the n-hexane extract showed no detectable antibacterial activity against any of the tested organisms. This finding suggests that the major antibacterial compounds in *Z. officinale* are predominantly polar phytochemicals that are more efficiently extracted by polar solvents such as methanol. The effectiveness of polar solvents in extracting antimicrobial compounds from ginger has also been reported in several recent phytochemical studies (Bashir *et al.,* 2015). Although the zones of inhibition produced by the extracts were smaller than those of the standard antibiotic ciprofloxacin, the results indicate that ginger possesses notable antibacterial activity that could contribute to the development of plant-based antimicrobial agents.

Table 1: Zone of Inhibition (mm) of Z. Officinale Extracts against the Test Bacterial Isolates

		Extract Concentration (mg/mL)				
Extracts	**Isolates**	**50**	**25**	**12.5**	**6.25**	**CPX (10 µg/ml)**
Methanol	*E. faecalis*	14.67 ± 0.44	8.33 ± 0.44	6.67 ± 0.44	0.00 ± 0.00	28.67 ± 0.44
	S. aureus	18.00 ± 0.00	12.00 ± 0.00	10.33 ± 0.44	0.00 ± 0.00	34.00 ± 0.67
	E. coli	14.33 ± 0.44	8.33 ± 0.89	0.00 ± 0.00	0.00 ± 0.00	23.00 ± 0.00
	P. aeruginosa	13.00 ± 0.67	6.67 ± 0.89	0.00 ± 0.00	0.00 ± 0.00	24.67 ± 0.44
Ethyl acetate	*E. faecalis*	9.67 ± 0.44	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	S. aureus	11.33 ± 0.44	9.67 ± 0.44	0.00 ± 0.00	0.00 ± 0.00	
	E. coli	8.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	P. aeruginosa	8.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
n-Hexane	*E. faecalis*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	S. aureus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	E. coli	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
	P. aeruginosa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	

CPX = Ciprofloxacin

The MIC and MBC values of the ginger extracts are summarized in Table 2. The methanol extract exhibited inhibitory activity against three of the test organisms. The lowest MIC value (12.5 mg/mL) was observed against *S. aureus*, indicating a relatively high susceptibility of this organism to the ginger extract. Higher MIC values (50 mg/mL) were recorded for *E. faecalis* and *E. coli*, suggesting moderate antibacterial activity. The bactericidal effect of the methanol extract was observed only against *S. aureus* with an MBC value of 50 mg/mL, while bactericidal activity was not detected against *E. faecalis* and *E. coli*. The ethyl acetate extract showed weak inhibitory activity solely against *S. aureus* (MIC = 50 mg/mL), whereas the n-hexane extract showed no detectable inhibitory or bactericidal activity. The higher susceptibility of *S. aureus* to ginger extracts observed in this study corroborates earlier findings indicating that ginger-derived phytochemicals, particularly gingerols and shogaols, exhibit significant inhibitory effects against Gram-positive pathogens (Sulieman *et al.,* 2024). These compounds can disrupt bacterial cell membranes, inhibit enzymatic processes, and interfere with microbial metabolic pathways (Sulieman *et al.,* 2024).

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the *Zingiber officinale* Extracts

Extract	**Isolates**	**MIC (mg/mL)**	**MBC (mg/mL)**
Methanol	*E. faecalis*	50.0	-
	S. aureus	12.5	50.0
	E. coli	50.0	-
	P. aeruginosa	-	-
Ethyl acetate	*E. faecalis*	-	-
	S. aureus	50	-
	E. coli	-	-
	P. aeruginosa	-	-
n-Hexane	*E. faecalis*	-	-
	S. aureus	-	-
	E. coli	-	-
	P. aeruginosa	-	-

- = Not detected

The qualitative phytochemical screening results (Table 3) revealed the presence of several bioactive secondary metabolites including alkaloids, glycosides, saponins, terpenoids, flavonoids, phenols, and anthraquinones in the methanol extract. Ethyl acetate extract contained most of these phytochemicals except alkaloids and tannins, whereas the n-hexane extract lacked several key metabolites. The rich phytochemical composition of the methanol extract may explain its superior antibacterial and antioxidant activities. Many of the detected phytochemicals are known to possess antimicrobial and antioxidant properties. Phenolic compounds and flavonoids can disrupt microbial membranes, inhibit nucleic acid synthesis, and interfere with bacterial energy metabolism (Donadio *et al.,* 2021). Recent studies have shown that the antimicrobial activity of ginger is largely attributed to phenolic compounds such as gingerols, shogaols, and paradols, which possess strong antimicrobial and antioxidant properties (Sulieman *et al.,* 2024). The absence of certain phytochemicals in the n-hexane extract further explains its lack of antibacterial activity, as non-polar solvents generally extract lipophilic compounds that may not possess strong antimicrobial properties.

Table 3: Phytochemical Compositions of the *Zingiber officinale* Extracts

Phytochemicals	n-Hexane	Ethyl acetate	Methanol
Alkaloids	-	-	+
Anthraquinonne	-	+	+
Glycoside	+	+	+
Saponins	+	+	+
Tannins	-	-	+
Terpenoids	+	+	+
Total flavonoids	+	+	+
Total phenol	+	+	+

+= Detected
-= Not detected

The quantitative analysis of phenolic and flavonoid contents (Table 4) revealed significant variation among the extracts. The methanol extract exhibited the highest total phenolic content (1907.67 ± 14.22 mg GAE/100 g), followed by ethyl acetate (1621.00 ± 25.33 mg GAE/100 g), while n-hexane showed the lowest phenolic content (803.67 ± 5.78 mg GAE/100 g). In contrast, the ethyl acetate extract showed the highest total flavonoid content (207.33 ± 7.56 mg QE/100 g), followed by methanol and n-hexane extracts. Phenolic compounds are widely recognized as important natural antioxidants because of their ability to donate hydrogen atoms or electrons to neutralize free radicals (Foti, 2007). Numerous studies have demonstrated a strong positive correlation between phenolic content and antioxidant activity in plant extracts (Piluzza and Bullitta, 2011; Makhafola *et al.,* 2016). Therefore, the high phenolic content observed in the methanol extract may significantly contribute to its superior antioxidant activity.

Table 4. Total Phenolic and Flavonoid Contents of the *Zingiber officinale* Extracts

Solvents	Total Phenolic (mg GAE/100 g)	Total Flavonoids (mg QE/100 g)
Methanol	1907.67 ± 14.22	152.33 ± 11.56
Ethyl acetate	1621.00 ± 25.33	207.33 ± 7.56
n-hexane	803.67 ± 5.78	71.67 ± 7.78

The antioxidant activity of the ginger extracts was evaluated using the DPPH radical scavenging assay (Table 5). The results showed that all extracts exhibited concentration-dependent radical scavenging activity. Among the extracts, the methanol extract demonstrated the highest antioxidant activity with an IC_{50} value of 30.12 µg/mL, followed by ethyl acetate (43.7 µg/mL) and n-hexane (45.6 µg/mL). However, the antioxidant activity of the extracts was lower than that of the standard antioxidant ascorbic acid (IC_{50} = 10.9 µg/mL).
The strong antioxidant activity of the methanol extract is consistent with its high phenolic content and diverse phytochemical composition (Takaidza *et al.,* 2018). Phenolic compounds and flavonoids are known to act as effective free radical scavengers by donating hydrogen atoms or electrons to stabilize reactive oxygen species (Kaurinovic and Vastag, 2019). Recent studies have also reported that ginger extracts possess significant antioxidant capacity due to the presence of phenolic acids and flavonoids, which contribute to the neutralization of free radicals and prevention of oxidative stress (Tohma *et al.,* 2017; Anwar *et al.,* 2020). The concentration-dependent increase in DPPH scavenging activity observed in this study further supports the role of ginger-derived phytochemicals as effective natural antioxidants.

Table 5: DPPH Radical Scavenging Activities of the *Zingiber officinale* Extracts

Concentration	n-Hexane	Ethyl acetate	Methanol	Ascorbic acid
50	50.96 ± 0.35	58.31 ± 0.21	70.70 ± 1.19	93.89 ± 0.84
25	36.59 ± 0.28	26.99 ± 0.34	46.07 ± 0.86	78.66 ± 0.26
12.5	15.59 ± 0.33	17.74 ± 0.15	29.86 ± 0.69	63.48 ± 0.04
6.25	6.79 ± 0.15	12.77 ± 0.06	15.75 ± 0.67	47.58 ± 0.24
3.13	4.82 ± 0.10	10.32 ± 0.09	13.02 ± 0.12	35.31 ± 0.09
1.57	3.12 ± 0.08	8.23 ± 0.12	10.33 ± 0.16	24.61 ± 0.02
IC50	45.6	43.7	30.12	10.9

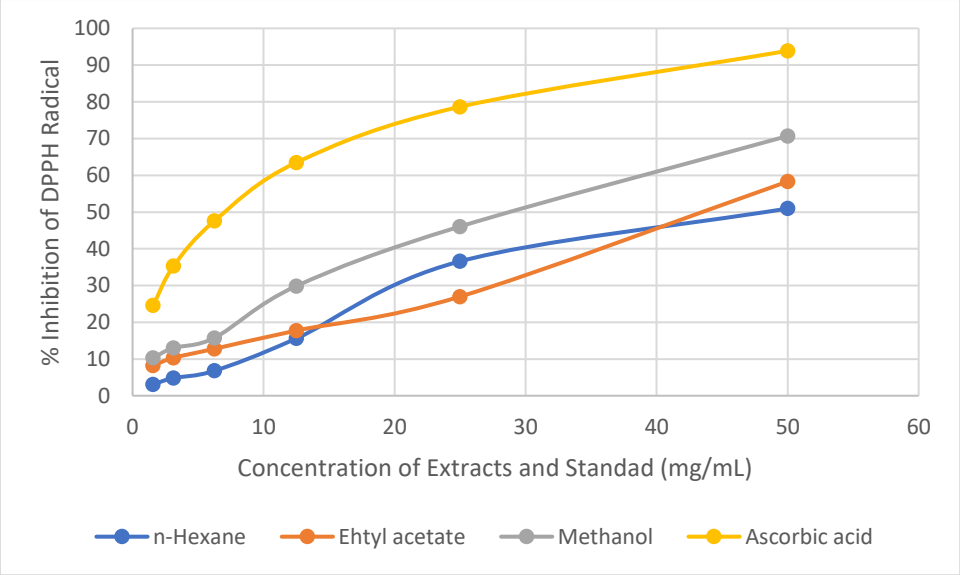

Figure 1: Dose Dependent DPPH Scavenging Activities of *Zingiber officinale* Extracts

CONCLUSION

The findings demonstrated that solvent polarity significantly influenced the extraction efficiency of bioactive compounds and their associated biological activities. Among the tested solvents, the methanol extract exhibited the strongest antibacterial activity against the tested bacterial isolates, particularly *Staphylococcus aureus*, indicating a higher susceptibility of Gram-positive bacteria compared with Gram-negative bacteria. Qualitative phytochemical screening revealed the presence of several bioactive secondary metabolites, including alkaloids, flavonoids, phenols, terpenoids, glycosides, and saponins, with the methanol extract containing the richest phytochemical profile. The antioxidant evaluation using the DPPH radical scavenging assay showed that all extracts exhibited concentration-dependent antioxidant activity. However, the methanol extract demonstrated the strongest radical scavenging activity among the tested extracts, which may be attributed to its higher phenolic content. Overall, the results of this study demonstrate that *Zingiber officinale* cultivated in Kaura Local Government Area of Southern Kaduna possesses significant antibacterial and antioxidant potentials. These findings support the traditional medicinal use of ginger and highlight its potential as a natural source of bioactive compounds that could be explored for the development of novel antimicrobial and antioxidant agents.

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