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Comparative Assessment of Antibacterial Activity of *Chromolaena odorata* Leaf Extracts against Selected Clinical Bacterial Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Author JOO designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors JAA and AKA managed the literature searches and analyses of the study. Authors SPEJ and KJS performed the statistical analysis. Authors CJ and FSO managed the microbiological aspect of the experimental. Authors QO, ISY and DD managed the extraction of the plant material. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This research is aimed to compare the antibacterial activities of *Chromolaena odorata* leaf extracts and some commonly used antibiotics against selected clinical bacterial isolates.
Place and Duration of Study: The study was conducted at the microbiology laboratory of the Nigerian Institute of Leather and Science Technology, Zaria between June and September, 2016.
Methods: The plant extracts were obtained using the cold extraction method and were concentrated by heating in water bath at 90°C for 48 hours. The identities of bacterial isolates collected were confirmed by standard biochemical methods. The plant extracts and bacterial

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isolates were stored at 4°C until required for use. The susceptibility of the bacteria to extracts was tested using the agar well diffusion method while the modified Kirby Baur agar discs diffusion method was used for the antibiotics susceptibility test. The antibiotics activity was subjected to MAR index analysis and the results noted.

Results: The results of this study showed that water had significantly higher percentage yield (13.21%) compared to the other extraction solvent (ethanol) with the yield of 10.45%. The results of our study indicated that the ethanolic extract was more effective in comparison with the aqueous extract. Although at 125 mg/mL, the both extracts were able to inhibit the growth of majority of bacteria tested. The antibiotic susceptibility profile analysis indicated that *Proteus mirabilis* and *Pseudomonas aeruginosa* had high level resistance to some of the tested antibiotics whereas *Bacillus subtilis* was resistant to only one of the tested antibiotics. Streptomycin was most active whereas Ofloxacin was less active. All the bacteria except *Bacillus subtilis* had MAR index of ≥ 0.2. *Bacillus subtilis* had 0.1.

Conclusion: The findings of this study show that *Chromolaena odorata* leaf extracts possess a good antibacterial activity against the tested bacteria. It is clear from this study that most of the pathogens were resistant to the tested antibiotics but susceptible to the plant extracts although at high MIC and MBC.

Keywords: Antimicrobials; alternative medicine; drug resistance; medicinal plants; phytomedicine.

1. INTRODUCTION

Modern medical science has been developed to a great extent but many rural people still depend on plant products and herbal remedies for treating their ailments. Some African countries are locally producing traditional medicines used for various diseases such as chronic diarrhoea, liver disorders, amoebic dysentery, constipation, cough, eczema, ulcers, hypertension, diabetes, malaria, mental health, and HIV/AIDS in order to improve people's access to medicines. Plant materials have been found to be active against infectious diseases, for instance, *Garcinia biflavonone* has been found to be active against a wide variety of microorganisms such as *Salmonella typhi*, *Escherichia coli, and Staphyloccocus aureus* [1]. It is also used in treatment of liver disorder and throat infection. Some extracts of green pepper, garlic, and onions have been noticed to inhibit the growth of *Shigella dysenteriae and Salmonella typhi*. *Pterocarpus santalinoide* are used as vegetables in food preparation and the leaves are as fodder for feeding livestock. *Senna alata* has been identified as a medicinal plant used in the cure of many ailments and diseases in many parts of the world and the leaves are taken orally as an effective laxative and are used in case of constipation [2]. However, fresh juice squeezed out from the leaves of *Chromolaena odorata* is used to stop bleeding and in wounds healing [3].

C. odorata leaf extracts in ethanol, methanol, and hexane have been reported to exhibit strong inhibitory effects against both Gram positive and Gram negative bacteria [4]. It has antibacterial activities against *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Neisseria gonorrhoeae* and antifungal activities against *Cryptococcus neoformans*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* [5].

Chromolaena odorata antibacterial properties is said to be associated with sinensetin and scutellaréinetetrametyl ether which were the two bioactive compounds identified in a study by Atindehou et al. [5] although these compounds have previously been identified in a chemical study by Barua et al. [6].

Considering the vast potentiality of plants as sources for antimicrobial drugs the present study is aimed to compare the antibacterial activities of *Chromolaena odorata* leaf extracts and some commonly used antibiotics against selected clinical bacterial isolates.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Chromolaena odorata (Siam weed) was obtained from Abuja, Nigeria and validated at the Herberium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The test organisms include *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia, Proteus mirabilis,* and *Escherichia coli*. The organisms were obtained

from the medical laboratory unit of Ahmadu Bello University sickbay and from the Department of Pharmaceutical Microbiology, Ahmadu Bello University, Zaria. The identities of the bacteria were confirmed by standard biochemical methods [7].

2.2 Preparation of the Leaves

The plant leaves were collected and washed to reduce microbial load to a large extent. The leaves were air-dried for five days. After sufficient drying, a warring industrial blender was used to crush the leaves to powder and then weighed.

2.3 Ethanolic Extraction

The ethanolic extraction was done by weighing 100 g of pulverized powdered leaves of the plant using an electronic weighing balance and weighed sample was soaked in clean 1000 mL conical flask containing 500 mL of ethanol. The mixture was stirred vigorously with a stirrer at intervals. After 72 h with interval stirring, the mixture was filtered using a clean filter paper (Whatman no 1 filter paper) into a clean beaker and the filtrate was concentrated to dryness by evaporation using a steam bath at 90°C for 48 h. The extracts obtained were stored in the refrigerator at 4°C as stated by Hanphakphoom et al. [4].

2.4 Aqueous (Cold Water) Extraction

For the aqueous extraction, 100 g of the pulverized powdered leaves was weighed and soaked separately into 500 mL conical flask containing 400 mL of distilled water. The mixture was stirred vigorously with stirrer. After 72 h interval stirring, the mixture was filtered using a clean filter cloth into a clean beaker and the filtrate was concentrated to dryness by evaporation using a steam bath at 90°C for 48 h. The standard extract obtained was stored in a refrigerator at 4°C until required for use [8,9].

2.5 Preparation of Extract for Anti-bacterial Testing

One gram of ethanolic extract of the pulverized leaves was mixed with 1 mL of water to yield 1000 mg/mL. This concentration was diluted using doubling dilution method and seven (7) dilutions were obtained including the stock concentration viz; 1000 mg, 500 mg, 250 mg, 125 mg, 62.5 mg, 31.25 mg, and 15.625 mg [10]. The same procedure was followed for the cold water extract.

2.6 Media Preparation

All the materials used were sterilized after being washed with detergent and rinsed severally with distilled water. The media used for culturing the organisms was Müller Hinton agar. 38 g of the medium was suspended in one litre of distilled water and autoclaved at 121°C for 15 minutes and allowed to cool to 50°C. The media was dispensed into Petri dishes and allowed to set [11].

2.7 Susceptibility Test Using Plant Extract

Müller Hinton agar plates were inoculated with respective test organisms which have been adjusted to the 0.5 McFarland scale corresponding to approximately 1.5 x 10^8 cfu/mL. Suspensions of the isolates were inoculated on Mueller Hinton agar plates using a sterile swab. The swab was streaked evenly over the surface of the medium to ensure confluent growth. The plates were allowed to dry for 15 minutes in an incubator. Wells were made on the plate using cork-borer leaving enough space in between wells to prevent the resulting zone of inhibition from overlapping. 0.5 mL of the different concentrations was added to the respective holes. The plates were incubated at 37°C for 18-24 h to observe the zone of growth inhibition produce by the extract [12]. The sensitivities of the bacterial species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of wells) on the agar surface and values <8 mm were considered as not active against the bacteria.

2.8 Antibiotics Susceptibility Profile of Bacteria

The antibiotics for this study included Amoxicillin (30 µg), Augmentin (30 µg), Erythromycin (10 µg), Ciprofloxacin (10 µg), Pefloxacin (10 µg), Ofloxacin (30 µg), Gentamicin (10 µg), Streptomycin (30 µg), Trimethoprim-Sulfamethoxazole (30 µg), and Chloramphenicol (30 µg). Antibiotic sensitivity test was carried out using the modified Kirby Bauer's agar discs diffusion technique. After the inoculation of test organisms into various agar plates, the plates were allowed to dry in an incubator for 15 minutes and antibiotic discs were then placed on

the agar plates using disc dispenser. The plates with the antibiotic discs were incubated at 37°C for 24 h to observe for the zones of inhibition produced by the antibiotics [11]. The sensitivities of the bacterial isolates to the antibiotics were determined by measuring the sizes of zones of inhibition (including the diameter of discs) on the agar surface around the discs, and values <8 mm were considered as not active against the bacteria.

2.9 Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of the extract was determined by doubling dilution. 1 g of each extract was dissolved in 1 mL of sterile water to obtain 1000 mg/mL. This 1000 mg/mL concentration was then diluted using the doubling dilution method in sterile distilled water to obtain concentrations of 1000 mg, 500 mg, 250 mg, 125 mg, 62.5 mg, 31.25 mg, 15.625 mg, and 7.513 mg. Each tube containing the extract was inoculated with 1 mL of standardized 0.5 McFarland suspensions of the bacteria and incubated at 37°C overnight. The tubes were observed for development or absence of turbidity.

2.10 Minimum Bactericidal Concentration

The minimum bactericidal concentration (MBC) of the extracts against bacteria was tested by streaking the surface of Müller Hinton agar plates with non-turbid tubes from the MIC. The plates were incubated overnight and were investigated for bacterial growth. The least concentration that inhibited the growth was considered as the MBC.

2.11 Multiple Antibiotic Resistance (MAR) Index Study

The MAR Index of each of the isolates was determined using the formula:

$$MAR\ Index = \frac{a}{b}$$

Where 'a' represents the number of antibiotics to which the isolate was resistant to and 'b' represents the number of antibiotics to which the isolate was subjected to Oko et al. [13]. The MAR indices of the isolates were calculated and noted.

3. RESULTS

Water and ethanol were the extraction solvents used in this study. The results showed that water showed significantly higher percentage yield (13.21%) compared to the other extraction solvent (ethanol) with the yield of 10.45%.

The results of the antibacterial analysis of the ethanolic extract indicated that all the bacteria tested were susceptible at the concentration of 125 mg/mL except *Pseudomonas aeruginosa* which was completely resistant to this concentration. *Klebsiella pneumoniae* and *Escherichia coli were intermediate* whereas *Proteus mirabilis* was however susceptible at 62.50 mg/mL as *S. aureus* was weakly sensitive (Table 1).

The most effective concentration of the aqueous extract was 250 mg/mL. *Staphylococcus aureus, Klebsiella pneumoniae,* and *Proteus mirabilis* were weakly sensitive to the extract at 125 mg/mL (Table 2). Out of the bacteria tested, *Staphylococcus aureus* was most sensitive to the aqueous plant extract.

The results of the MIC and MBC analyses of the ethanolic extract indicated that *Pseudomonas aeruginosa* had the highest MBC of 500 mg/mL making it less susceptible to the extract when compared with other bacteria. *Bacillus subtilis, Klebsiella pneumonia* and *Escherichia coli* all recorded a uniform MBC of 250 mg/mL. *Proteus mirabilis* recorded an MIC and MBC of 250 mg/mL (Fig. 1).

The analyses of the aqueous extract for MIC and MBC determination showed that *Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae,* and *Echerichia coli* had a uniform high MBC and MIC of 1000 mg/mL and 500 mg/mL respectively making it less effective when compared with ethanolic extract (Fig. 2).

Proteus mirabilis and *Pseudomonas aeruginosa* had high level resistance to some of the tested antibiotics while *Bacillus subtilis* was resistant to only one of the tested antibiotics. Streptomycin was most active whereas Ofloxacin was less active (Table 3).

The results of the MAR index analysis indicated that all the isolates except *Bacillus subtilis* had MAR index of ≥ 0.2. *Bacillus subtilis* had 0.1 (Table 4).

4. DISCUSSION

Water and ethanol were the extraction solvents used in this study. The results revealed that water showed significantly higher percentage

yield (13.21%) compared to the other extraction solvent (ethanol) with the yield of 10.45%. The extract yield was associated with the extraction solvent. This is evident by water being more polar organic solvent having higher yield than ethanol. This is similar to those reported by Hanphakphoom et al. [4]. In their study, water and methanol solvents showed significantly higher yields compared with the other extraction solvents (ethanol and hexane) for the plant leaf and stem. The lowest percentage yield reported was obtained from *Chromolaena odorata* root extracted with hexane solvent. Furthermore, hexane solvent gave a significantly lower percentage yield for all plant parts compared with water, ethanol, and methanol solvents. In both studies, it was observed that yields were proportional to polarity of solvent.

Table 1. Antibacterial activity (zone of inhibition, mm) of *C. odorata* ethanolic leaf extract

Bacteria	Concentrations in mg/mL						
	1000	500	250	125	62.50	31.25	15.63
Staphylococcus aureus	26	22	22	16	08	00	00
Pseudomonas aeruginosa	23	16	14	00	00	00	00
Bacillus subtilis	24	22	18	16	00	00	00
Klebsiella pneumoniae	22	18	16	12	00	00	00
Proteus mirabilis	26	22	22	18	16	00	00
Escherichia coli	22	18	14	13	00	00	00

All measurements are in millimetre (mm)

Table 2. Antibacterial activity (zone of inhibition, mm) of *C. odorata* aqueous leaf extract

Bacteria	Concentrations in mg/mL						
	1000	500	250	125	62.50	31.25	15.63
Staphylococcus aureus	26	18	16	12	00	00	00
Pseudomonas aeruginosa	22	12	13	00	00	00	00
Bacillus subtilis	24	14	10	00	00	00	00
Klebsiella pneumoniae	22	14	12	11	09	00	00
Proteus mirabilis	24	17	14	11	00	00	00
Escherichia coli	23	12	08	00	00	00	00

All measurements are in millimetre (mm)

Table 3. Antibiotics susceptibility profile of test bacteria species (zone of bacterial inhibition, mm)

Bacteria	Antibiotics									
	STR	SXT	CHL	CPX	AMX	AUG	GEN	PEF	OFX	ERY
Staphylococcus areus	11	12	32	20	14	32	16	24	20	00
Pseudomonas aeruginosa	18	14	00	28	24	00	12	30	00	22
Bacillus subtilis	30	30	20	24	32	24	22	24	24	00
Klebsiella pneumonia	22	22	00	22	24	00	18	36	00	20
Proteus mirabilis	18	00	00	20	00	00	00	00	00	00
Escherichia coli	20	24	00	00	34	30	00	20	00	20

Key: STR= Streptomycin, SXT= Septrin, CHL= Chloramphenicol, CPX=Ciprofloxacin, AMX=Amoxicillin, AUG= Augmentin, GEN= Gentamicin, PEF=Pefloxacin, OFX= Ofloxacin, ERY= Erythromycin

Table 1. Multiple antibiotic resistance index analysis of isolates

Bacterial species	Antibiotics	MAR index
Staphylococcus areus	AMX,ERY	0.2
Pseudomonas aeruginosa	CHL,AMX,AUG,GEN,OFX	0.5
Bacillus subtilis	ERY	0.1
Klebsiella pneumonia	CHL,OFX	0.2
Proteus mirabilis	SXT,CHL,AMX,AUG,GEN,PEF,OFX,ERY	0.8
Escherichia coli	CHL,CPX,GEN,OFX	0.4

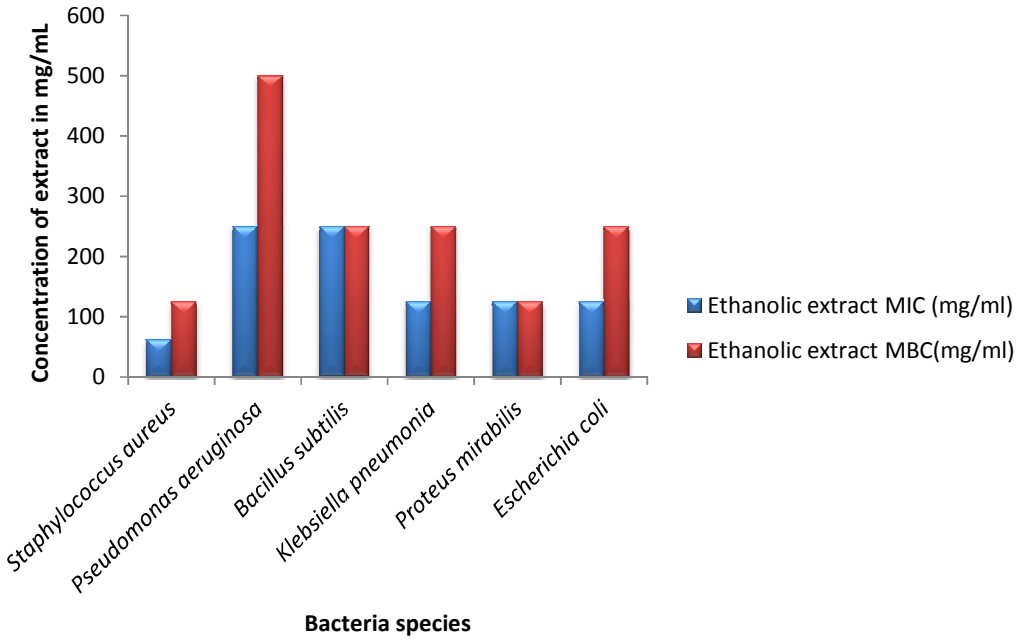

Fig. 1. Results of minimum inhibitory concentration of the ethanolic extract

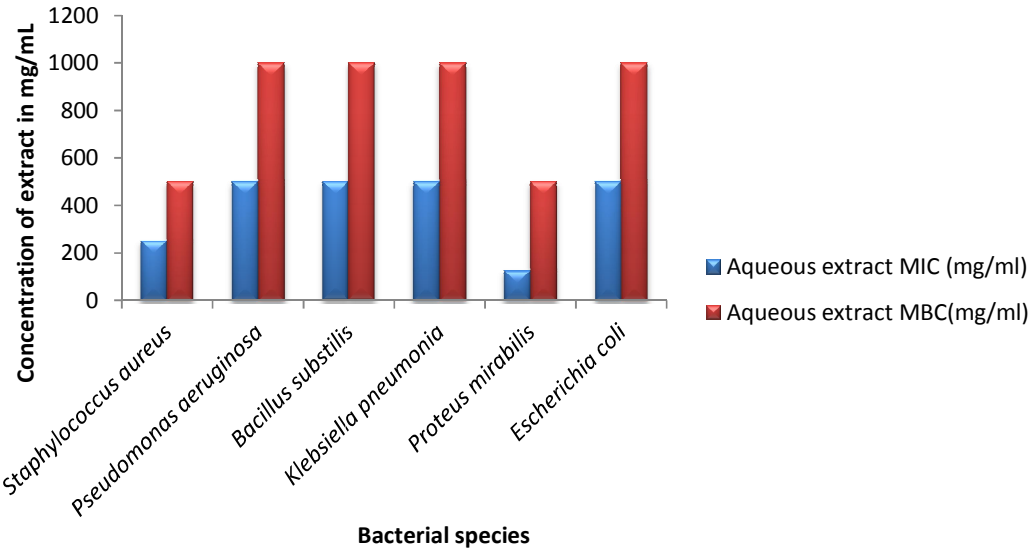

Fig. 2. Results of minimum bactericidal concentration of the aqueous extract

In this study, our results showed effectiveness of ethanolic extract against the studied bacteria particularly *Staphylococcus aureus* and *Proteus mirabilis.* The aqueous extract at 125 mg/mL was able to inhibit 50% of the bacterial population studied (Table 2). The extract was equally active against Gram positive and Gram negative bacterial species studied. These views confirm the reports of Hanphakpaoom et al. [4] that the plant leaf extract contains some vital metabolites (phenol and flavonoid) that have a broad antibacterial activity. This observation is in agreement with the views of Sharma et al. [14] who reported that extracts or phytoconstituents derived from various parts of medicinal plants for prevention and cure of several diseases provide therapeutic modalities with broad spectrum antimicrobial activities against various

pathogenic microorganisms. The use of *C. odorata* in traditional medicine for the treatment of skin infections and diarrhoea is not out of place since scientific evidence [12,15] exist to justify its utilization. This is also evident in our current study where bacteria tested in this study showed measurable zones of inhibition against both Gram positive and Gram negative bacteria.

The ethanolic leaf extract of *C. odorata* was found to have better antibacterial activity than the aqueous extract (Tables 1 and 2), the zones of bacterial inhibition range from 16-26 and 9-26 for ethanolic and aqueous leaf extracts respectively. This is in agreement with the reports of Hanphakphoom et al. [4] who reported better antibacterial activity with ethanolic leaf extract against bacteria tested in their study in comparison with the aqueous leaf extract. Unlike the reports of Hanphakphoom et al. [4], our study recorded some measurable zones of inhibition with the aqueous extract (Table 2). This may be due to the high concentration of the extract used in our study.

Although the results of the MIC and MBC of the extracts were high at 500 mg/mL and 1000 mg/mL respectively for the aqueous extract against majority of the bacteria studied, it can still be harnessed to formulate antibacterial agents for treating some maladies caused by these bacteria. The report of the ethanolic extract showed that it has a lower MIC and MBC of between 125 mg/mL and 500 mg/mL respectively for most of the bacterial species. Our study has indicated that the ethanolic leaf extract of *C. odorata* is good. This is supported by the findings of Naidoo et al. [8], who reported that methanol extracts derived from the leaves inhibited all Gram positive bacteria and including one Gram-negative bacteria, *E. coli.* Results obtained by Atindehou et al. [5] demonstrated that *C. odorata* displayed an antibacterial activity ranging from 0.156 to 1.25 mg/mL against *Klebsiella oxytoca, Salmonella enterica, Shigella sonnei* and *Vibrio cholerae*.

In this study, bacterial isolates were mostly intermediate or resistant to the tested antibiotics *in vitro* (Table 3). This phenomenon may be due to genetic changes since antimicrobial resistance occurs naturally over time [13]. However, the misuse and overuse of antimicrobials is accelerating this process. In many places, antibiotics are overused and misused in people and animals, and often given without professional oversight. Examples of misuse include when they are taken by people with viral infections like colds and flu, and when they are given as growth promoters in animals and fish [16]. The attendant consequences of antimicrobial resistance include increase cost of health care with lengthier stays in hospitals and more intensive care. The bacteria studied in this research were all clinical isolates with majority recording MAR index of greater than 0.2 (Table 4). This is in agreement with the views of Oko et al. [13] who reported that high MAR indices indicate high risk source of contamination of antibiotics. This is also in agreement with Manegabe [17] who supported that antimicrobial resistant-microbes are found in people, animals, food, and the environment (in water, soil, and air). They can spread between people and animals, and from person to person. Poor infection control, inadequate sanitary conditions, and inappropriate food-handling encourage the spread of antimicrobial resistance [17]. The results of the MAR indices analyses indicated that all the isolates except *Bacillus subtilis* were from antibiotic laden sources especially being that they were isolated from medical samples. The organisms may have been exposed to these antibiotics through indiscriminate use of the drugs or wrong prescription. It could also be that low dosages (< MIC) of the drugs were used over time.

5. CONCLUSION

This study has proven the effectiveness of *Chromolaena odorata* leaf extract against some medically important isolates. This plant has promising medicinal benefit and could be developed to produce potent antimicrobial agents that may be used for the treatment of some common and antibiotics resistant pathogens. It is clear from this study that most of the pathogens were resistant to the tested antibiotics but susceptible to the plant extracts although at high MIC and MBC.

6. RECOMMENDATION

From the results of this research, it is recommended that the plant be studied extensively to determine their biomedical importance and possible formulation into antibacterial agents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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