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Research Article Antioxidant Phytochemical Screening and Antimicrobial Activity of *Ficus exasperata* Against Pathogens in Nigeria

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Abstract

Background and Objective: Development of microbial resistance to drugs has led scientists to study the role of natural substances such as medicinal plants with antimicrobial and antioxidant activity compared to synthetic drugs. Aim of present study was to evaluate antimicrobial and antioxidant activities of *Ficus exasperata* leaves against selected human pathogens and also screen medicinal plant for phytochemical activity in comparison to the synthetic drugs. **Materials and Methods:** *Ficus exasperata* was screened for phytochemicals by standard methods, it was also observed for their antimicrobial activity by agar well diffusion methods. Antioxidant activities were assessed on the basis of the scavenging effect of the 2, 2-dipheny-1-pircryl hydrazyl (DDPH) free radicals activity. **Results:** Phytochemical screening showed presence of steroids, tannins, saponins, anthocyanins, phenols, anthraquinones, flavonoids, terpenoids and alkaloids. Highest concentrations of extracts resulted in highest antimicrobial activity. Ethyl acetate extracts showed highest antimicrobial activity against pathogens which was followed by methanolic extract. Lowest minimum inhibitory concentration was also shown by ethyl acetate, followed by methanol. As concentration of plant extract increased, antioxidant activity also increased. Ethyl acetate was able to show highest activity of antioxidants compared to other solvents. **Conclusion:** Based on broad spectrum activity of *F. exasperata* extracts as revealed by this research supports its usage as natural antimicrobial and antioxidant compared to the synthetic drugs.

Key words: Ficus exasperata, phytochemicals, microbial resistance, human pathogens, natural antimicrobial, antioxidant activities

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Health related illnesses in developing world are overcome by the use of medicinal plants as well as their products due to their accessibility, availability and affordability. Many studies in developing countries proved that these medicinal plants have fertility regulating properties^{1,2}.

The *F. exasperata* is an ever green tree which can be as tall as 20 m and is found in countries like Senegal, Cameroon, Sudan, Uganda, Cameroon, Tanzania, Natal (South Africa), Madagascar Central and East Africa as well as Nigeria mainly in the Northern Eastern part. *Ficus exasperata* is abundant in the Savannah regions, especially along river banks and marshy areas at an altitude of up to 1100 m. The leaves are displayed spirally, limb is oval or ellipse and the roots are fibrous³. The roots of this plant are used to cure cough, gastritis, urinary disorders and haemorhoids as well bark of the tree is used to relieve pain and heal wounds. The leaves are considered to be abortificient⁴. Fresh shoots are boiled to prepare soup like solution which is given to breast feeding women to facilitate breast feeding.

In Africa, extract of medically important plants are used as anthelmintic and a purgative⁵. Reactive oxygen species (ROS) are released during metabolic processes of many cells such as mitochondrial respiration, metabolism of Xenobiotics by cytochromes P450, inflammation and phagocytosis which damages lipids, proteins and nucleic acids which can cause oxidative stress⁶⁻⁹. Oxidative stress may be one of the causes of diseases like cardiovascular, rheumatoid arthritis, neuro-degenerative diseases, diabetes mellitus and cancer. Some of these medicinal plants are being used for the treatment of such diseases as well as repair oxidative damage¹⁰. Phytochemicals of these medicinal plants possess an efficient ROS scavenging capability which can protect cells against oxidative damage caused due to the release of these ROS molecules^{8,11}.

In Cameroon, *Ficus* exasperata is traditionally used to cure sterility/infertility whereas the leaves are used as anthelmintic and purgative³. Watcho *et al.*¹² found that this plant induced uterotonic effect by stimulating prostaglandin secretion. Watcho *et al.*¹³ studied the effect of this medicinal plant for reproduction in female rats, which supported its popular use to treat sterility and infertility problems in women. The antibacterial, antioxidant, fibroblast growth and wound healing properties of *Ficus exasperata* have been reported¹⁴. Stem bark infusion of the plant is used in Nigeria and Ghana by traditional healers to treat sores and ulcer. Due to a number of properties of *Ficus exasperata* for treatment of many diseases, present study was carried out to (1) Screen

phytochemicals present in *Ficus exasperata* (2) Determine the antimicrobial and antioxidant activities of the plant (3) Determine the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the plant extract.

MATERIALS AND METHODS

Collection of plant materials and micro-organisms: Plants were collected from a farm yard at Olorunsogo, Bode-Olude Abeokuta, Ogun state. Plants were air dried for 1 week. Test organisms used in this study were all clinical methicillin resistant isolates (pure cultures) collected from Department of Medical Microbiology and Parasitology, Sacred Heart Hospital, Lantoro, Abeokuta. This study was performed during February-June, 2017.

Preparation of plant extracts: Leaves were washed then air dried for 7 days and grinded into powder using electric blender and weigh into 3 different containers for methanol, ethyl acetate and aqueous extract. About 25 g of the plant was added into 150 mL of each extract such as water, acetone and ethanol and were allowed to stay for 48 h. Plant extracts were then filtered using Whatman No. 1 filter paper and were concentrated using rotary evaporator.

Quantitative photochemical screening: Phytochemicals such as phenols, alkaloids, steroids, anthocyanins anthraquinones, tannins, tarpenoids, saponins and flavonoids were screened following the standard procedures as described by Odebiyi and Sofowora¹⁵.

Determination of antimicrobial activity (agar well diffusion method): Antimicrobial activities of extracts, positive control (Ciprofloxacin and Fluconazole) and negative control (methanol and ethyl acetate) against all the test isolates were evaluated by agar well diffusion method¹¹. Plates were incubated at 37°C for 24 h for bacterial pathogens and 28°C for 48 h for fungal pathogens. Antimicrobial activity was determined by measuring the zone of inhibition in millimeter (mm).

Minimum Inhibitory Concentration (MIC) assay: Tolerance of microbial strains against different concentrations of plant extracts were carried out in broth inoculated with 10^8 cells mL⁻¹ as described by Ochei and Kolhatkar¹⁶. Test tubes were kept at 37 ± 2 C for 24 h and were monitored regularly for bacterial growth/survival. The highest plant

extracts concentrations which inhibited bacterial growth were considered as minimum inhibitory concentration. Ciprofloxacin was used as positive control for bacteria while fluconazole was used for fungi. The solvents were used as negative control.

Determination of minimum bactericidal and fungicidal concentration : Sub-culturing was made from each bacterial and fungal well showing no growth in the MIC wells onto blood agar plates for bacteria and sabouraud dextrose agar plates for fungi. Following incubation at 37 °C for 24 h in case of bacteria and 28 °C for 48 h for fungi, the plates were examined for growth of bacterial and fungal colonies. Lack of growth indicates that the *C. citratus* extracts are having bactericidal and fungicidal potential, while as growth indicates bacteriostatic and fungi static activity.

Antioxidant activity (DPPH (2, 2-Dipheny-1-Pircryl Hydrazyl) free sadical scavenging activity): The antioxidant activities of plant extracts were assessed on the basis of the scavenging effect of the 2, 2-dipheny-1-pircryl hydrazyl (DDPH) free radicals activity as described by Davies ¹⁷.

Statistical analysis: Significant difference among the treatments were calculated using Duncan's multiple range test at p < 0.05. Values indicate Mean \pm SD of three replicates.

RESULTS

Phytochemical constituents of *Ficus exasperata* **extract:** The Table 1 shows phytochemical compounds/constituents of the extracts of the leaves of *Ficus exasperate*. Among the three extracts, ethyl acetate showed significantly highest extraction of the phytochemicals, which was followed by the methanolic extracts while as aqueous extract showed least extraction of the phytochemicals. Among all the phytochemicals screened, significantly highest yield of tannins was obtained by ethyl acetate extract (12.52 mg g⁻¹) which was followed by methanol (11.70 mg g⁻¹). Second only to tannin was saponins showing yield of 2.32 mg g⁻¹ by ethyl extract and 1.22 mg g⁻¹ by methanolic extract. Anthocyanins and anthraquinones are the phytochemicals whose yield was least when extracted with methanol, ethyl extract or aqueous extract.

Antimicrobial activity of *Ficus exasperate* extract: Antimicrobial activity of all the three extracts is shown in Table 2. All the extracts studied showed significantly higher antimicrobial activity against all the pathogens used in this study. Significantly highest antimicrobial activity was shown by ethyl acetate, followed by methanolic extracts. As the concentration of the extract increased, antimicrobial activity of the extract also increased significantly. Concentration of 200 mg mL⁻¹ showed significantly highest toxicity against all the pathogens used. Significantly highest antibacterial activity of ethyl acetate extract of F. exasperata was shown against Klebsiella pneumonia having the mean zone diameter of 17 mm at a concentration of 200 mg mL⁻¹ followed by methanolic extract 15 mm compared to control (Ciprofloxacin) having a zone diameter of 25 mm at the same concentration. This was followed by ethyl extract against S. aureus showed second highest significant antibacterial activity (16 mm zone at a concentration of 200 mg mL⁻¹), followed by methanolic extract (14 mm zone at 200 mg mL $^{-1}$), compared to the control (Ciprofloxacin) which showed a zone of inhibition of 25 mm at the same concentration.

Methanolic and ethyl acetate extract significantly showed highest actifungal activity against *T. ruburum* which was followed by *C. albicans* whereas both extracts showed less antifungal activity against *A. fumigates.* Methanolic and ethyl acetate extract of *F. exasperata* showed antifungi activity of 18 and 17 mm, respectively against *T. rubrum* at a concentration of 200 mg mL⁻¹ compared to 25 mm zone when exposed to fluconazole at the same concentration. Methanol and ethyl acetate showed least antifungal activity of 8 and 13 mm, respectively towards *A. fumigates* at a concentration of 200 mg mL⁻¹ compared to fluconazole which showed a zone of inhibition of 26 mm at the same concentration. Methanol and ethyl acetate alone did not show any antimicrobial activity and isolates were resistant to methanol and ethyl acetate.

Assay of minimum inhibitory concentration (MIC): Among all the extracts tested against bacteria and fungi, ethyl acetate extract showed significantly lowest value of MIC against them (Table 3). All the extracts in this study showed lowest MIC towards *S. aureus* and *Klebsiella pneumonia* while as highest MIC was observed towards *P. aeruginosa*. Among the three extracts of *F. exasperata* tested in this study, ethyl acetate showed lowest MIC of 16.6 mg mL⁻¹ followed by methanoilc extract (20.8 mg mL⁻¹), while the highest MIC of 66.6 mg mL⁻¹ was recorded for aqueous extraction against *S. aureus* compared to the control (ciprofloxacin) having mean MIC of 5.2 mg mL⁻¹.

Ficus exasperata extract also showed significantly lowest MIC values towards *Candida albicans* and *T. rubrum*

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Table 1: Phytochemica	l analysis (mg g ⁻	1) of leaf extracts of	Ficus exaperata
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Parameters	Methanol extracts (mg g ⁻¹)	Ethyl acetate extract (mg g ⁻¹)	Aqueous extract (mg g ⁻¹)
Steroids	0.21±0.02 ^b	0.32±0.01ª	0.16±0.01ª
Alkaloids	0.84±0.01 ^b	0.92±0.01ª	0.22±0.01 ^c
Phenols	0.21±0.01 ^b	0.81±0.01ª	0.14±0.01 ^c
Tannins	11.70±0.20 ^b	12.52 ± 0.10	0.27±0.04 ^c
Flavonoids	0.45 ± 0.01^{b}	0.76±0.02ª	0.33±0.01 ^c
Saponins	1.22 ± 0.03^{b}	2.32±0.04ª	1.07±0.01
Terpenoids	0.14±0.01 ^b	0.20±0.01ª	0.13±0.01 ^b
Anthocyanins	0.03±0.01ª	0.50±0.01ª	0.01 ± 0.01^{a}
Anthraquinones	0.03±0.02ª	0.32±0.01ª	0.28±0.02ª

^{abc}Means values with different superscripts along the same row are significantly (p<0.05) different by Duncan's Multiple range test. Values are mean of three replicates ±Standard Deviation

Table 2: Antimicrobial activity of *Ficus exasperate* extract (mg mL⁻¹)

		Zones of inhibition (mm)						
		Bacterial species			Fungal species			
Extracts	Concentration	 ۲ عبيتمبيد	K nneumonia		Calbicans	T rubrum	A fumicatus	
Mothanolic oxtract	(IIIg IIIL) 25	9. 20/203	8+1 0 ^b	8±0.6 ^b	10+1 16	10+0.63	0+0.04	
Methanolic extract	23	0 <u>1</u> 1.0	0 - 1.0	0 <u> </u>	10 - 1.10	10±0.0	0 - 0.0	
	50	$10 \pm 1.5^{\circ}$	12±0.6ª	10±1.1°	13±1.16ª	12±0.6°	/±0.5°	
	100	12±1.5⁵	15±0.6ª	11±1.0 ^b	15±1.53ª	15±1.0ª	9±0.7℃	
	200	14±1.5 ^b	15±1.5 ^b	12±0.6°	15±0.58 ^b	18±0.9ª	8±0.5ª	
Ethyl acetate extract	25	9±0.6 ^{bcd}	11±1.0ª	8±1.0 ^d	11±0.5 ^{ab}	10±0.5 ^{abc}	8±0.6 ^{cd}	
	50	13±1.0 ^{ab}	14±1.5ª	11±0.6°	12±0.6 ^b	13±0.6 ^{ab}	10±0.6°	
	100	15±1.0 ^{ac}	16±1.5ª	13±0.5 ^{cd}	14±0.6 ^{bcd}	15±0.7 ^{ab}	12±1.0 ^d	
	200	16±1.1 ^{ab}	17±1.5 ^{ab}	15±0.6 ^{ab}	15±0.7 ^ь	17±1.0ª	13±1.0℃	
Aqueous extract	25	7±0.5ª	7±0.4ª	$0\pm0.0^{\text{b}}$	8±1.0ª	7±0.4ª	$0\pm0.0^{ m b}$	
	50	8±0.6 ^b	10±0.5ª	$0\pm0.0^{\circ}$	9±1.00 ^b	10±0.7ª	0±0.0°	
	100	10±0.7c	12±1.2 ^{ab}	8±0.5 ^d	11±0.6 ^b	13±0.9ª	7 ± 0.4^{d}	
	200	12±1.0 ^{bc}	13 ± 1.0^{ab}	10±0.7°	14±0.8 ^{ab}	15±1.2ª	10±1.5°	
Ciprofloxacin	200	25±1.0ª	25±1.0ª	26±2.3ª	ND	ND	ND	
Fluconazole	200	ND	ND	ND	25±1.0 ^{ab}	25±2.1 ^b	26±0.5ª	
Methanol	200	NA	NA	NA	NA	NA	NA	
Ethyl acetate	200	NA	NA	NA	NA	NA	NA	

Methanol and ethyl acetate alone did not show any antimicrobial activity and isolates were resistant to methanol and ethyl acetate.^{abc}Means with different superscripts along the same row are significantly (p<0.05) different by Duncan's Multiple range test. Values are mean of three replicates ±Standard deviation. ND: Not determined, NA: No activity

Table 3: Minimum inhibitory concentration of *Ficus exasperata* (mg mL⁻¹)

	Extracts			Control	
Test organisms	Methanol Ethyl acetate		Aqueous	Ciprofloxacin	Fluconazole
Bacterial species					
S. aureus	20.8±2.2 ^b	16.6±2.3 ^{bc}	66.6±8.8 ^b	5.2±0.7ª	ND
K. pneumonia	20.8±2.2 ^b	12.5±0.9 ^{bc}	100.0 ± 10^{a}	6.2±0.8ª	ND
P. aeruginosa	25.0±2.5 ^b	25.0±1.9 ^{ab}	100.0±9.8ª	6.2±0.7ª	ND
Fungal species					
C. albicans	17.0±1.4 ^b	12.5±0.8 ^{bc}	50.0±5.3 ^{bc}	ND	6.2±0.8ª
T. rubrum	12.5±1.1 ^b	10.4±0.6°	41.6±4.4	ND	4.1±0.5 ^b
A. fumigatus	21.0±1.4 ^b	33.0±3.4ª	100.0±9.4ª	ND	6.2±0.6ª

^{abc}Means with different superscripts along the same row are significantly (p<0.05) different by Duncan's Multiple range test. Values are mean of three replicates ± Standard Deviation. ND: Not determined

while as all the extracts showed highest MIC values against *A. fumigates*. Ethyl acetate extraction had lowest mean MIC of 10.4 mg mL⁻¹ followed by methanolic extract (12.5 mg mL⁻¹) against *T. ruburum*. Second lowest MIC values of 12.5 mg mL⁻¹ and 17 mg mL⁻¹ was shown

against *Candida albicans* by ethyl acetate and methanol, respectively. Highest mean MIC of 33.0 and 21 mg mL⁻¹ was recorded for ethyl acetate and methanol, respectively against *A. fumigates* compared to the control (fluconazole) having MIC of 6.2 mg mL⁻¹.

	Extracts		Control		
Test organisms	Methanol	Ethyl acetate	Aqueous	Ciprofloxacin	Fluconazole
Bacterial species					
S. aureus	33.3±4.4 ^b	25.0±1.8 ^b	100.0±9.7ª	12.5±1.0ª	ND
K. pneumonia	41.6±4.3 ^b	25.0±2.3 ^b	100.0±9.6ª	12.5±1.2ª	ND
P. aeruginosa	50.0±4.7 ^b	50.0±5.4ª	100.0±8.9ª	12.5±0.9ª	ND
Fungal species					
C. albicans	25.0±2.5 ^b	25.0±2.0 ^b	100.0±8.4ª	ND	12.5±0.8ª
T. rubrum	25.0±2.2 ^b	25.0±2.5 ^b	83.3±7.7ª	ND	12.5±1.0ª
A. fumigatus	83.3±7.8 ^b	66.6±7.2ª	100.0 ± 10.2^{a}	ND	12.5±1.1ª

Table 4: Minimum bactericidal and fungicidal concentration (mg mL⁻¹)

Table 5: Effect of plant extract on DPPH % (2, 2-dipheny-1-pircryl hydrazyl) free radical scavenging activity

Concentration (mg mL ⁻¹)	Methanol	Ethyl acetate	Aqueous extract		
25	18.03±0.9ª	23.1±1.5ª	13.2±0.7ª		
50	31.06±2.5 ^b	35.5±2.7 ^b	24.5±1.4 ^b		
100	61.30±5.6°	68.2±6.5 ^c	33.7±2.2 ^c		
200	70.80±7.5°	82.7±8.6°	45.3±4.6 ^d		

In control (0% concentration, there was no anti-oxidant activity)

Minimum bactericidal/fungicidal concentration assay: Ethyl

estate and methanolic extract showed significantly lower minimum bactericidal and fungicidal effect against all the organisms tested (Table 4). Ethyl acetate and methanolic extract showed the lowest BMC of 25.0 and 33.3 mg mL⁻¹ respectively against *S. aureus* while the highest mean BMC 100.0 was recorded by aqueous extraction. Control (ciprofloxacin) showed BMC value of 12.5 mg mL⁻¹. Ethyl acetate and methanolic extract showed the lowest MFC (25.0 mg mL⁻¹) while the highest mean MFC 100.0 was recorded by aqueous extraction against *Candida albicans* while as control (fluconazole) showed MFC value of 12.5 mg mL⁻¹.

Antioxidant activity [DPPH (2, 2-Diphenyl-Pircryl Hydrazyl) free radical scavenging activity)]: Ethyl estate and methanolic extract showed significantly higher antioxidant activity is shown in Table 5. Antioxidant activity of all the plant extracts increased upon increase in the concentration of the extract. Highest activity was observed at 200 mg mL⁻¹ of all the plant extract used.

The antioxidant value for ethyl extract was found to be 23.1, 35.5, 68.2 and 82.7%, respectively for 25, 50, 100 and 200 mg mL⁻¹ followed by methanolic extract which showed an antioxidant value of 18.03, 31.06, 61.3 and 70.8%, respectively at the same concentration whereas aqueous extract showed anti-oxidant values of 13.2, 24.5, 33.7 and 45.3% at concentrations of 25, 50, 100 and 200 mg mL⁻¹ plant extract, respectively. As it could be observed, for each concentration of plant extract, ethyl acetate extract showed the highest anti-oxidant effect, followed by that of methanolic extract.

DISCUSSION

Qualitative phytochemical screening of the ethyl acetate, methanolic and aqueous extract of *F. exasperata* is shown in Table 1. Result of the present study confirmed ethyl acetate extraction to be the best and more effective solvent compared to methanol and aqueous extracts as it recovered highest concentration of phytochemicals. The higher concentrations of phytochemicals is in accordance with the study of Wani *et al.*⁸, Lawal *et al.*¹⁸ and Shrestha *et al.*¹⁹. This study is also in accordance with the report of Awala *et al.*²⁰ and Ismail *et al.*²¹.

Phytochemicals are plant molecules that are not directly involved in plant's growth but for other secondary activities such as protection against pest, pigmentation, abiotic stress etc²². These chemicals in the medicinal plants have been reported in several studies to be responsible for the healing potentials²³. In this study, a wide range of phytochemicals which show antimicrobial activities hence, collaborative or synergic action of these phytochemicals is responsible for the antimicrobial activity of *Ficus exasperata*.

Comparative study shows that ethyl acetate extract of *F. exasperata* had greater potency against the test organisms than methanolic extract and aqueous. Moreover, 200 mg mL⁻¹ concentration of ethyl acetate extract had high inhibition rate against the microbial growth of all the test organisms studied which was followed by the methanolic extract had lesser potency compared to ethyl acetate extract on the test organisms, this is in contrary to the work of Lawal *et al.*²⁴, who reported that methanolic extract had high inhibition rate against the microbial growth.

Furthermore, Barku *et al.*²⁵ and Khurm *et al.*²⁶ reported the antibacterial activity of the plants extract may be due to the presence of metabolic toxins and broad spectrum antimicrobial compounds that may act against bacteria. Antimicrobial activity in the present study may be due to the presence of phytochemicals in the plant extracts. These results are in agreement with the results of Baba and Malik¹⁰, who studied that the presence of phytochemicals in *Arisaema jacquemontii* Blume were responsible for the high antimicrobial activity against pathogens.

Minimum Inhibitory Concentrations (MIC) is used by diagnostic laboratories to established resistance or to determine *in vitro* activity of antimicrobials¹⁰. The basic qualitative measures of the *in vitro* activity of antimicrobials are the minimum inhibitory concentration (MIC) or minimum bactericidal/fungicidal concentration (MBC/MFC). Ethyl acetate extract exhibited significant MIC on all the test organisms ranging from the lowest MIC value of 10.4 mg mL⁻¹ to the highest MIC of 33.0 mg mL⁻¹. Therefore, the ethyl acetate extract exhibited lowest mean MIC of 10.42 mg mL⁻¹ towards *T. rubrum* which is in accordance with the study of Okwulehie and Akanwara²⁷ that recorded MIC value of 8.0 mg mL⁻¹.

From the present experimental data, it is revealed that ethyl acetate extract has the highest effect on scavenging free radicals (23.1-82.7%). The antioxidants are believed to play a very important role in the body defence system against reactive oxygen species or free radicals, which are harmful by product generated during the aerobic activity of normal cell^{28,9,6-8}. Further investigations are exploited to address issues involving mechanism of toxicity shown by the plant extract against the pathogens, also level of damage to the cell structure by using scanning electron microscope, florescent microscopy as well as transmission electron microscopy. Interaction within cells and oxidative damage to the cell will also be evaluated.

CONCLUSION

In this study, *F. exasperata* extract showed presence of various phytochemicals. Ethyl acetate and methanolic extract showed effective antimicrobial and antioxidant activity compared to aqueous extracts. Also, the antibacterial and antifungal activity of the leaf extracts against the test organisms also supports its exploitation in traditional system of medicine practice.

SIGNIFICANCE STATEMENT

This study discovered a wide range of phytochemicals of *Ficus exasperate* which show antimicrobial activities and ethyl acetate and methanol of *Ficus exasperate* could be beneficial antimicrobial agents against methacillin resistant microbes and could also protect the body against the oxidative damage. This plant extract has a novel antimicrobial activity which was compared to the synthetic drugs which confirmed the novelty of this research compared to the research done earlier.

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