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ANTIBACTERIAL EFFICACY OF FICUS CARICA FRUIT AGAINST FOLLICULITIS AND DERMATITIS BACTERIA AND TOXICITY STUDY IN RATS MODEL

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Abstract
The aim of this study was to find the antibacterial activity of *Ficus carica* fruit against bacteria isolated from dermatitis and folliculitis. Thus the alarming situation of antibacterial resistance can be battled by using secondary metabolites of plants. *Ficus carica* fruit was collected, identified and authenticated. After washing, drying and grinding, extracts were made in solvents like ethanol, methanol, petroleum ether and acetone. Samples were collected from dermatitis and folliculitis patients, proceeded for characterization and bacterial isolation. The isolates were subjected to extracts by disk diffusion and well diffusion method to analyze ZoI, MIC and MBC. The maximum extract yield was obtained from methanol. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were isolated. FCFM showed 15 to 16mm ZoI while FCFA and FCFE were observed with 11mm. FCFM was found safe while performing in vitro study on rats. FCFM was found to be the rich source of secondary metabolites and also proven to be the great source of drug for antimicrobial resistant bacteria. It could be used in therapeutic skin formulations to treat skin infections.

Key words: Antibiotics, Antibiotic Resistance, Herbal Medicine, *Ficus carica*, Antibacterial Potential

Introduction

The increasing demand of medicinal plants is due to its therapeutic value, to generate revenue, widely acceptance and affordability in developing countries. Medicinal plants are still practiced in most Asian countries due to therapeutic active ingredients and in Pakistan more than 2000 species have been identified whereas 400 are extensively used as folk medicines (Munir *et al*., 2022). Over the generations, this trend is known as traditional medicine used in the treatment of mental and physical illness. According to the data analysis, out of 100,000 publications, 50% plants are grouped under Medicine and Pharmaceutic category while 11% are under the category of Biological and agricultural sciences (Salmerón *et al*., 2020). Simultaneously, these medicinal plants were also identified as food and basic analytical techniques for example, paper chromatography and instrumentation development was invention of last seven decades (Fitzgerald *et al*., 2019).

Using standardized analytical techniques, new bioactive compounds are isolated from plants which offer direct approach against pathogenic bacteria in the era of drug resistance pathogens. As drug

resistance issue is emerging worryingly due to absurd, extensive, irregular and unselective antibiotic use. This concern highlighted the importance of natural bioactive chemicals isolation from plants that may inhibit the growth of microbes by different mechanisms (Vaou *et al*., 2021). These natural bioactive compounds provide the antimicrobial activity even alone or in combination, therefore, in the battle of drug resistance, extracts or medicinal plants are the best solution (Fazly *et al*., 2018). The mechanism of action is multi-target in case of synergism, which may involve the suppression of resistance mechanism of bacteria, enhancing the bioavailability of drug, increasing the resorption rate and reducing toxicity (Ruddaraju *et al*., 2020).

Ficus carica is a tree which shed their foliage annually and belong to family Moraceae. It is native to mediterranian basin where it was cropped as food, due to its adaptive ability to different soils and climate, it grows around the world. Its fruit is an excellent source of nutrients and antioxidants (Martínez *et al*., 2022). It also propagates in tropical and sub-tropical areas of india where it has different names like in Tamil called atti pazham, telgu-athi pallu, and in hindi-anjeer or gullu. Traditionally, fig is used for various disorders like scabies, ringworm and leukoderma, inflammations, paralysis, diabetes, asthma and cough. It is a great source of organic acids, fatty acids, phenolic and volatile compounds (Badgujar *et al*., 2014). Fig products are used for treatment of atopic dermatitis, skin warts and cervical cancer (Hajam *et al*., 2022).

Hence, the prime objective of this study was to investigate the antibacterial property of *Ficus carica* fruit against bacteria isolated from dermatitis and folliculitis. As well as the toxicity effects was also evaluated by in vivo experiments.

Material and Methods

The isolation and characterization of bacteria, plant extraction, phytochemical analysis by biochemical tests, GC-MS and HPLC was conducted at Institute of Molecular biology and Biotechnology, The University of Lahore from July 2021 to March 2023. Sigma-Aldrich, Germany (commercially available) solvents and standard were utilized for additional purification.

Collection of plant Material

The fresh fruit of *Ficus carica* were collected from botanical garden of Punjab University Lahore, Pakistan. Fruit were identified and authenticated as *Ficus carica* by Prof. Abdul Rehman Niazi, Botany Department, Punjab University, Lahore. Voucher specimen were deposited to herbarium of same University with number LAH# 08922.

Extract Preparation

Fruits were washed with tap water, dried in oven at 40℃ followed by shade dried for 20 days (Soni *et al*., 2014). Dried materials were then grinded into powder. Extracts were prepared using four different solvents ethanol, methanol, acetone and petroleum ether. 25g plant powder was dipped in 300ml solvent each., and left for 5 days with continuous mild agitation (Felhi *et al*., 2017). After filtration with filter paper, extract was processed to rotary evaporator under reduced pressure, to obtain the crude extract. The extracts obtained were calculated for yield percentage, then solubilized with 10% DMSO and stored in refrigerator at 4℃ (De Zoysa *et al*., 2019). Different concentrations of extract were prepared as 1000, 2000 and 5000µg to check antibacterial potential against different bacteria.

Collection of Sample and identification of bacteria

To collect sample from patients of dermatitis and folliculitis, different areas were selected and washed with normal saline to prevent contamination. In case of folliculitis, scalp, chin, axilla and leg were identified whereas for dermatitis forearm, neck, popliteal and antecubital fossa were selected. Swab were moistened with sodium chloride and Tween -20 (0.9% and 0.1% respectively) and samples were collected in Z stroke manner, labeled then transported to the laboratory (Ogai *et al*., 2018).

In laboratory, according to the directions of Sigma manufacturer, Nutrient agar media plates were prepared and streaked with sample to isolate the colonies. Moreover, motility test was executed followed by Gram staining and were streaked again on differential and special media to study morphology. Finally, by performing biochemical tests separate bacteria were identified. The selected colonies were subculture on Nutrient agar and stored in refrigerator (Khayyira *et al*., 2020).

Qualitative Antibacterial assay

Disk-Diffusion Method: Initially, the disk diffusion method was used to assess the antibacterial activity of the *Ficus carica* fruit extracts. the Muller Hinton agar and blood agar plates were prepared and streaked with isolated test colonies of bacteria (De Zoysa *et al*., 2019). By using the WhatmanNo.1 filter paper, 6mm discs were made and then soaked in 1000, 2000 and 5000 (µg /ml) concentrations of *Ficus carica* fruit extracts (formerly prepared). The extract soaked filter paper were then placed on the Muller Hinton agar and blood agar plates. Plates were incubated at 37℃ for 24 hrs and after incubation the zone of inhibition (ZoI) were measured as the diameter of growth free zone including the disc. The sensitivity of strains was also measured against ampicillin as positive control, and 10% DMSO as negative control. The diameter more than 10mm zone of inhibition was considered significant for the test result (Adamczak *et al*., 2020 and Benmaghnia *et al*., 2019).

Minimum inhibitory Concentration (*MIC*)

Ficus carica fruit extracts with positive disk diffusion results were further proceeded to determine the MIC and MBC (Minimum Bactericidal Concentration) by standard procedure. 100mg of each *Ficus carica* fruit extract was dissolved in DMSO (1ml), serial dilutions of extracts were prepared at conc. 12.5, 25 and 50. Muller Hinton broth was used to prepare bacterial inoculum and was calibrated to 0.5 McFarland standard. The bacteria were added to plant extracts and turbidity in solution was measured after 24 hours of incubation at 37℃. The tubes that showed no turbidity were documented as MIC (Mishra *et al*., 2017). The lowest concentration of extract dilution showing no visible growth was documented as MBC (Wasihun *et al*., 2016).

In vivo Safety study of extracts

The present study was carried out on 75 healthy (age 6-7 weeks and weight 90-100g) albino rats acquired from University of veterinary and Animal Sciences, Lahore Pakistan. The study was first approved by the departmental Bioethics, Biosafety and Biosecurity Committee (BBC) of The University of Lahore-Pakistan with reference IMBB/UoL/21/1037. Rats were first examined to rule out the presence of disease and were housed in separate cages with britrat food and water supply for 4 days to accustomed to laboratory (Nassan *et al*., 2015). The guidelines of National committee for Research Ethics in Science and Technology (NENT 2018) were followed for handling the laboratory animals.

To evaluate the safe dose and adverse effects of *Ficus carica* fruit methanol (FCFM) extract on vital organs, acute and subchronic study (Zeiger., 2003) carried out and for this with few modifications in guidelines provided by, Organization for Economic Cooperation Development (OECD No.48) were used. Total 75 albino rats were used, which were further divided into 05 groups of 15 rats each: Group A: negative control group given water and food only, Group B: for acute toxicity dosed with 200 mg/kg twice orally for 14 days, Group C, D, E: for sub chronic toxicity experimented for 28 days. Group C was dosed with 10mg/kg twice orally, Group D-30mg/kg twice orally, Group E-100mg/kg twice orally. All groups were housed in steel wire mesh cage separately at room temperature. All groups were fasted overnight, prior to dosing.

After immediate dosing, at 6 hrs. interval and 24 hrs., skin, mucous membrane, salivation, lacrimation and change in respiratory and behavior pattern were noted (Nassan *et al*., 2015 and Zeiger., 2003).

After given days, rats were anaesthetized and blood samples were collected via cardiac puncture for hematological studies, whereas, for histopathological studies rats were dissected and liver and kidney were separated, cleaned and preserved in 10% formalin for at least 24hours and then processed to

prepare slides. Paraffin sections were prepared, stained with Hematoxylin and eosin stain to examined microscopically. The liver, kidney and heart weighed for any change relevant to control group (Bonam *et al*., 2019).

Statistical analysis

Results were expressed as mean ± SEM of each group. Data statistical analyses were achieved by using One-way ANOVA and Tukey-test. The level of significance was set at $P < 0.05$.

Results

Yields percentage and extract colors

Ficus carica fruit extracts were obtained with ethanol, methanol, acetone and petroleum ether solvents and the maximum yield percentage was acquired from Methanol 52% (and minimum from petroleum ether 1.6 %. Table 1 contains the percentage values of *Ficus carica* fruit extracts in different solvents and their color.

Table 1: Percentage yield of *Ficus carica* fruit extracts using different solvents

Plant Name	**Plant Part (25 g)**	**Solvent (300ml)**							
		Ethanol		**Methanol**		**Acetone**		**Petroleum ether**	
		Extract Quantity (g)	**Extract Yield (%)**	**Extract Quantity (g)**	**Extract Yield (%)**	**Extract Quantity (g)**	**Extract Yield (%)**	**Extract Quantity (g)**	**Extract Yield (%)**
Ficus carica	Fruit	11	44	13	52	5	20	0.4	1.6

Bacterial strains isolation

Both Gram positive and Gram negative strains were isolated from skin swab samples of dermatitis and folliculitis using CLSI (Clinical and Laboratory Standard Institute) microbiological guidelines. Gram positive strains identified as *Staphylococcus aureus* and *Staphylococcus epidermidis* however, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were Gram negative. Table 2 describes the characteristic of isolated strains.

Table 2: Results observed by Gram staining, Motility test and Biochemical tests on isolated colonies

Lab Identification	**Colony-I**	**Colony-II**	**Colony-III**	**Colony-IV**	**Colony-V**
Gram Staining	Positive Cocci	Positive Cocci	Negative Rod	Negative Rod	Negative Rod
Motility Test	Negative	Negative	Negative	Positive	Positive
Biochemical tests					
Catalase	Positive	Positive	Positive	Positive	Positive
Coagulase	Positive	Negative	Negative	Negative	Negative
Oxidase	Negative	Negative	Negative	Positive	Negative
Indole	Negative	Negative	Negative	Negative	Positive
Urease	Positive	Positive	Positive	Negative	Negative
VP	Positive	Positive	Positive	Negative	Negative
MR	Positive	Negative	Negative	Negative	Positive
Citrate	Positive	Negative	Positive	Positive	Negative
Lactose	Positive	Positive	Positive	Negative	Positive

Nitrate reduction	Positive	Positive	Positive	Positive	Negative
H_2S	Negative	Positive	Negative	Negative	Negative

Out of 50 strains, 55 % were Gram Negative rods and 45 % were Gram Positive cocci. After Gram staining motility test was performed and on the basis of it Gram Positive Cocci were found non motile while among Gram Negative isolates few isolates were found non-motile (18.3%) and others were motile (36.7 %).

Antibacterial Potential

By using disk diffusion method, antibacterial potential of *Ficus carica* fruit extracts were calculated against isolated Gram positive and negative bacteria. The zone of inhibition was the antibacterial potential of extracts expressed in Table 3. The MIC and MBC values were measured for FCFM and are given in table 4.

Table 3: Assessment of Zone of Inhibition of *Ficus carica* fruit extracts against Gram positive and Gram Negative bacterial isolates by disk diffusion method

Bacteria	**Test Conc. (µg/ml)**	**FCFE**	**FCFM**	**FCFA**	**FCFPe**	**Positive Control** (Ampicillin 10µg / ml)	**Negative Control** (DMSO)
Staphylococcus aureus	**1000**	7	**9**	6	-	16mm	0
	2000	9	**13**	9	7		
	5000	11	**15**	11	8		
Staphylococcus epidermidis	**1000**	8	**10**	-	-	19mm	0
	2000	12	**13**	7	6		
	5000	14	**16**	9	8		
Klebsiella pneumoniae	**1000**	7	**8**	-	-	18mm	0
	2000	9	**12**	8	-		
	5000	11	**14**	9	7		
Pseudomonas aeruginosa	**1000**	6	**9**	6	-	13mm	0
	2000	9	**12**	7	6		
	5000	11	**14**	9	8		
Escherichia coli	**1000**	6	**9**	-	-	15mm	0
	2000	8	**12**	6	-		
	5000	10	**15**	9	6		

The extracts showed dose response ZOI and the significant result was measured in case of *Ficus carica* fruit methanol (FCFM) extract against both Gram positive bacteria and *Escherichia coli* (Gram negative) 15mm to 16mm ZOI at 5000µg/ml concentration. Although, at same concentration, 11mm ZOI was observed against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* while 14mm for *Staphylococcus epidermidis* in case of *Ficus carica* fruit ethanol (FCFE) extract. *Ficus carica* fruit acetone (FCFA) extract was only significant against *Staphylococcus aureus* with 11mm zone of inhibition. FCFM extract had showed best results which were equal to ampicillin for *Staphylococcus aureus* and *Escherichia coli* while 1mm greater for *Pseudomonas aeruginosa*. 10% DMSO was used as negative control and no zone of inhibition was observed.

Fig. 1: Antibiotic sensitivity testing for *Staphylococcus epidermidis* on blood agar at various concentrations. Zone of Inhibition was measured and showed dose dependent relationship and maximum zone was observed, maximum ZoI i.e. 16mm was measured with FCFM extract at 5000µg/ml.

Table 4: MIC (Minimum Inhibitory concentration) and MBC (Minimum bactericidal Concentration) values *Ficus carica* fruit methanol (FCFM) extract on isolates

Bacteria	**Test Conc. (µg / ml)**	**FCFM**
Staphylococcus aureus	**MIC**	3570
	MBC	9850
Staphylococcus epidermidis	**MIC**	3500
	MBC	9100
Klebsiella pneumoniae	**MIC**	5155
	MBC	9250
Pseudomonas aeruginosa	**MIC**	4250
	MBC	9750
Escherichia coli	**MIC**	6753
	MBC	>10000

In Vivo study results

On the basis of best MIC and MBC values, *Ficus carica* fruit methanol (FCFM) extract was processed further to in vivo acute and subchronic toxicity evaluation. The hematological profile of the rats is given in table 5. Organ weight of control and treatment groups was expressed in figure 2. Histopathological study was performed on liver and kidney tissue their observations are given in table 6 and microscopic visuals in figure 3. No significant changes in treatment groups were observed compared to the control group and no death observed. Moderate amount of inflammation and architectural changes observed in liver tissue at dose 200mg/kg dose, while minor parenchymal change observed in kidney tissue at the same dose. In case of 10,30, and 50 mg doses, no significant changes were observed.

Table 5: Hematological parameters studied on FCFM treated subchronic and acute toxicity group

Extract	**Hematological parameter**	**Sub-chronic**			**Acute**
		10mg/kg	**30mg/kg**	**50mg/kg**	**200mg/kg**
FCFM	ALT (U/L)	45.6 ± 0.5	46 ± 0.01	34.6 ± 0.5	35 ± 0.01
	AST (U/L)	168 ± 1.1	169 ± 0.01	184 ± 1.1	185 ± 0.01
	Creatinine (mg/dl)	0.6 ± 0.01	0.6 ± 0.01	0.6 ± 0.01	0.6 ± 0.01

	TP (g/dl)	6.5 ± 0.1	6.6 ± 0.01	7.2 ± 0.1	7.3 ± 0.01
Control	ALT (U/L)	49 ± 0.01			
	AST (U/L)	169 ± 0.5			
	Creatinine (mg/dl)	0.6 ± 0.01			
	TP (g/dl)	5.9 ± 0.05			

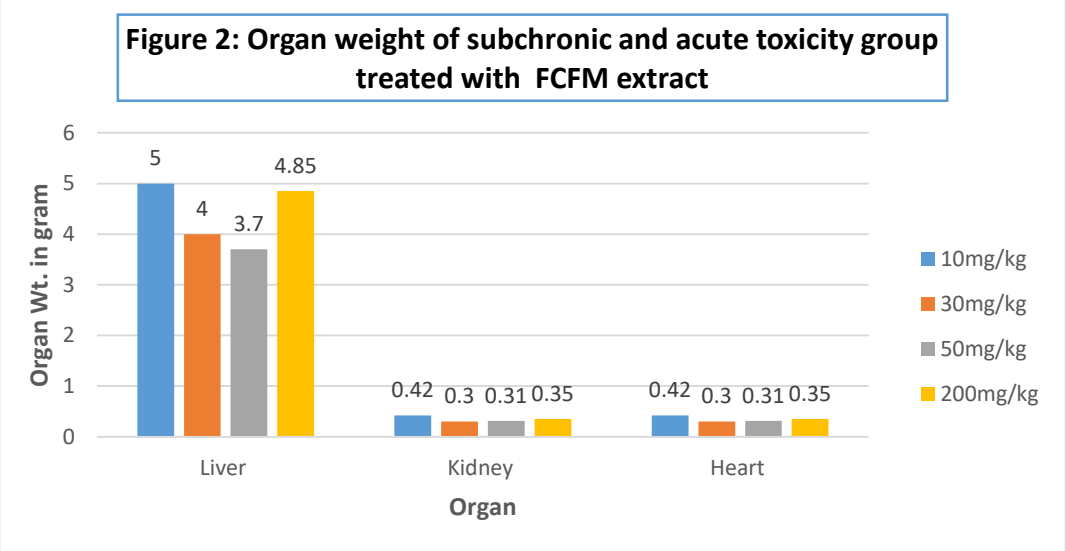
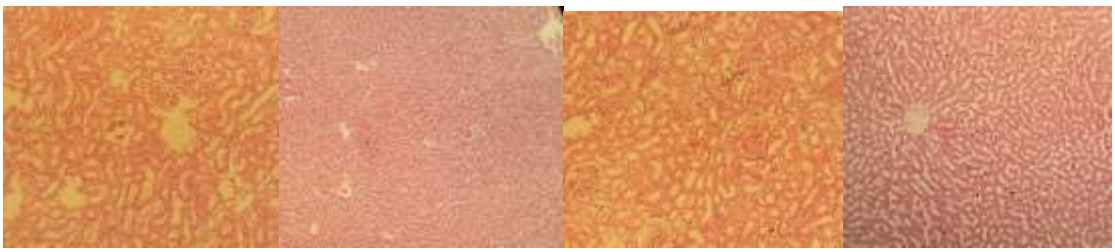


Table 6: Histopathological Study on liver and Kidney tissue of rats treated with FCFM after sub chronic and acute toxicity

Extract	**Dose (mg /kg)**	**Liver tissue Histopathology**						
		Ballooning	**Steatosis**	**Cholestasis**	**Necrosis**	**Inflammation**	**Fibrosis**	**Architectural changes**
FCFM	10	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-
	200	-	-	-	+	++	+	+
Control		-	-	-	-	-	-	-

		Kidney tissue Histopathology						
		Renal parenchyma	**Tubules**	**Glomerular infiltrates**	**Vacuolar degeneration**	**Cortical segment**	**Medullary Segment**	**Architecture**
FCFM	10	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-
	200	+	-	-	+	-	+	+
Control		-	-	-	-	-	-	-

"-' Normal, + mild effect, ++ moderate effect.



(A) **(B)** **(C)** **(D)**

Fig.3 : A- is the histological image of liver tissue treated with 50mg/kg FCFM extract, B-liver tissue treated with 200mg/kg FCFM extract, C- renal tissue treated with 50mg/kg FCFM extract, D-renal tissue treated with 200mg/kg FCFM extract**.**

Discussion

The dried fig has been used as edible due to its nutritious contents which includes carbohydrates, minerals, vitamins and phenolic compounds (Jeong *et al*., 2001). The presence of abundant secondary metabolite in fresh and dried fruits as anthocyanin, triterpinoids, coumarins, and hydrocarbonds had been observed. The fruit and bark parts of Ficus carica were found to be the precious source of anthocyanin pigments and mostly aglycon and perargonidin end products (Mawa *et al*., 2013). Ficus *carica* leaves extract in water contains organic acids like oxalic acids, malic acid, shikimc acid and fumaric acid. It's a rich source of phenolic acids also such as qurecitin-3-O-glucoside, rutinoside, bergapten and caffeolyquinic acid (Oliveira *et al*., 2009 and Liu *et al*., 2011). Its methanolic extract contains courmarins that exhibit strong nematocidal activity within 72 hours (Oliveira *et al*., 2010).
Fruit latex of fig inhibits the properties of Human papilloma virus as rapid growth and invasion by downregulating the receptor proteins p16, E6 and E7 in cervical cancer (Ghanbari *et al*., 2019). Both the water and methanol extract has strong potential to inhibit the enzymatic activity of alpha glucosidase, amylase, butyrylcholinestrase and acetylcholinesterase and act as antioxidant. While the methanolic extract showed moderate antibacterial activity against *Staphylococcus aureus* and *Escherchia coli* (Ergül *et al*., 2019).
The aqueous and methanolic extract of dried fig was more sensitive to *Citrobacter freundii* but resistant to *Listeria innocua* and the silver nanoparticles of fig extract had shown excellent antibacterial action against *Escherichia coli* and *Staphylococcus aureus* as compared to impenem (Logaranjan *et al*., 2012). The presence of flavonoids and phenolic compounds in fig for instance cerebrosides, steroids, triterpenes and ceramides increases its therapeutic importance for the treatment of diabetes, ulcer, vomiting, asthma, menstrual pain, scabies and gonorrhea. The psoralen and daidzein like furanocoumarins in its leave juice has magnificent results to treat vitiligo. Its hairy root has antilesishmanial effect. Fig extracts are very useful in relieving the symptoms of atopic dermatitis as compared to cortisone (Veberic *et al*., 2008).
The present study on *Ficus carica* fruit showed that its fruit extracts in different solvents had moderate antibacterial activity but the methanol extract indicated the best activity against bacteria isolated from dermatitis and folliculitis, both Gram positive and Gram negative bacteria with 15 to 16mm zone of inhibition. Its effect is in dose dependent relation and in vivo safety evaluation had also depicted the positive results. This study is narrow, however, pharmacodynamics and kinetic profile needed to be evaluated.

Conclusion:

Through this study, it is revealed that *Ficus carica* is an important medicinal plant with great therapeutic potential. Its phytochemical composition makes it favorable remedy against bacterial disease which occur due to direct infection or in response of secondary infection. By adding fig fruit in skin care products would be a beneficial for new industry and for human being. As well as it may be suitable alternative of antibiotics to counter antibiotic resistance.

Conflict of interest statement:
The authors declare that there are no conflicts of interest.

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