



## Antibacterial and Antifungal Activity of *Litsea monopetala* Leaves on Selected Pathogenic Strains

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### Authors' contributions

This work was carried out in collaboration between all authors. Author MSU designed the study, wrote the protocol and managed the analyses of the study. Author MFH carried out laboratory experiments and managed the literature searches under supervision of author MSU. Author MAI performed statistical analysis and prepared the draft of the manuscript. Author MSU reviewed the scientific contents of the manuscript. All the authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** To evaluate the antibacterial and antifungal activity of some solvent extracts of *Litsea monopetala* (LM) leaves.

**Study Design:** Examination of antibacterial and antifungal activity of petroleum ether fraction (PEF), chloroform fraction (CLF), ethyl acetate fraction (EAF) and crude methanol extract (CME) of *Litsea monopetala* (LM) leaves.

**Place and Duration of Study:** Department of Pharmacy, Southeast University, Dhaka-1213, Bangladesh, from September to November 2014.

**Methodology:** Fresh LM leaves were extracted with methanol followed by fractionation. Antibacterial and antifungal activities of the crude extract were determined by using the Agar disk

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diffusion method against gram positive, gram negative bacterial and fungal strains.

**Results:** All tested plant extracts, showed varying zones of inhibition against bacteria and fungi tested. The zone of inhibition for bacteria was found to be in the range from 5 to 15 mm and 5 to 13 mm for fungi. The CME and EAF of the methanolic extract of LM has greater antibacterial activity against all tested gram positive and gram negative bacteria compare to other fraction. CME and EAF has also strong antifungal activity against tested fungi except *Candida albicans* in which CLF showed maximum antifungal activity.

**Conclusion:** The present study shows that LM leaves possess an excellent source of natural antibacterial and antifungal agents which could be developed in the treatment of bacterial and fungal diseases.

**Keywords:** *Litsea monopetala*; antibacterial activity; antifungal activity; pathogenic strains.

## 1. INTRODUCTION

Medicinal plants are the basis of complementary and alternative medicine [1]. They are the richest natural sources of medicinal constituents used for the prevention or treatment of many diseases [2]. The search for medicinal values of different plants has attracted increasing interest throughout human history [3]. Ethno-botany is an effective way to discover modern medicines [4]. Some of the currently available pharmaceuticals as for example, aspirin, opium, digoxin and quinine are derived from plants that have a long history of use as complementary and alternative medicine (CAM) [5]. Asia represents an extensive antiquity of human dealings with the nature for the use of the natural pharmacopoeia (i.e., drug) [6]. As an integral part of the culture most of the developing countries have implemented CAM practice [7]. Today for primary health care need as stated by WHO (World Health Organization), 80% of the population of developing countries use CAM [8].

Uninvited guest (i.e., pathogenic microorganism) have always been considered as a major cause of morbidity and mortality in humans. The most common pathogenic gram positive bacterial such as *Bacillus*, *Staphylococcus aureus* and gram negative bacteria such as *Escherichia coli*, *Salmonella*, *Shigella dysenteriae* are responsible for food poisoning and produce abdominal pain, vomiting, diarrhea, fever etc. The rate of antibiotic resistance in *Escherichia coli* is hastily increasing, especially for fluoroquinolones, third- and fourth-generation cephalosporins. *Pseudomonas aeruginosa* strain produces infections in hospitalised patients, immunocompromised hosts and patients with cystic fibrosis and has revealed trends of increasing antimicrobial resistance, including carbapenem resistance and multidrug resistance. The genus *Candida* and *Aspergillus* are

pathogenic fungi that may cause to mucosal and skin infections or to deep-seated mycoses of almost all inner organs, especially in immunocompromised patients. At the present time, there are some effective antifungal agent's shows increasing resistances against these pathogenic fungi [9].

Medicinal plants represent a rich source of antimicrobial agents. There is also an urgent need to search for a new antimicrobial compound with novel mechanisms of action because there have been an alarming increase in the incidence of new infections diseases, as well as the development of resistance to the antibiotics in current clinical trials. Plant derived drugs proved to be relatively safe and dependable even in long-term use, where synthetic drugs are always feared in chronic cases [10].

The plant *Litsea monopetala* (LM) commonly known as Bara-kukurchita, Mendaphuri, Sukurja, Urujja, Akorma, Akarma, Lalkhori, Huoria and belonging to the family Lauraceae [11]. LM is mainly distributed in Western outer Himalayas, India, West Malaysia, Thailand and Burma [12]. LM is also widely distributed throughout the forests of Chittagong, Chittagong hill tracts, Sylhet and Sal forests of Gazipur, Madhupur, Dinajpur as well as villages throughout the Bangladesh. This plant is a small tree up to 18 m tall, leaves are 7.5-23 cm long, elliptic-oblong, usually rounded at both ends, pubescent beneath [11]. Traditionally the bark of this plant is used as nerves and bone tonic, stomachache, stimulant, analgesic and antiseptic. In fact bark is used as established herbal formulation in Pakistan and India. The water extract of the bark is given with sugar to treat diarrhea and dysentery. Powder of the bark is applied to body for pains arising from blows or bruises or from hard work. The leaves are great sources of

several phytoconstituents such as alkaloids, carbohydrates, tannins, flavonoids, steroids and reported to possess antioxidant, antihyperglycemic, antimicrobial, thrombolysis, anti-diarrheal and anti-inflammatory activities [13].

The present study was conducted to estimate the antibacterial and antifungal activities of petroleum ether fraction (PEF), chloroform fraction (CLF), ethyl acetate fraction (EAF) and crude methanol extract (CME) of LM leaves.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

All of the chemicals used in this study were analytical grade and purchased from Active Fine Chemicals Ltd., Bangladesh.

### 2.2 Collection and Identification of Plant Materials

The fresh leaves of LM were collected from Comillah, Bangladesh, in September, 2014 and identified by expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. Accession number: DACB-35517 for LM.

### 2.3 Drying and Grinding of Plant Materials

The fresh leaves of the plants were first washed with water to remove adhering dirt. Then fruits were cut into small pieces, sun dried for 10 days and finally dried in an oven at temperature not more than 50°C for better grinding. After drying, the entire portions were ground into coarse powder by a grinding machine and stored in an airtight container for further use.

### 2.4 Extraction and Fractionation of Plant Materials

Powdered sample having a weight of 250 g was taken in an amber colored glass bottle and soaked in 500 ml of 95% methanol at 25°C. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filter paper. Then the filtrate was concentrated with a rotary evaporator under reduced pressure at 50°C temperature to give crude extracts. An aliquot of the concentrated methanol extract was fractionated by petroleum ether, chloroform and ethyl acetate. Concentrated extracts and

different fractions were stored until further use and yield value of these were recorded.

## 2.5 Antimicrobial Activity

### 2.5.1 Test microorganism

Microorganisms used in this study were five gram positive bacteria strains *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Sarcina lutea*, *Staphylococcus aureus*; five gram negative bacteria strains *Pseudomonas aeruginosa*, *Vibrio minicus*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Escherichia coli* and three fungal strains *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* were obtained from the microbiology lab of the Department of Pharmacy, Southeast University, Dhaka.

### 2.5.2 Preparation of inoculums

The colony suspension method was used to prepare the inoculum of the test organisms. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi that were incubated without agitation for 24 hrs at 37°C and 25°C respectively. The bacterial and fungal strains were adjusted to a turbidity of 0.5 McFarland standards (approximately  $10^8$  CFU/ml for bacteria and  $10^5$  or  $10^6$  CFU/ml for fungi) with the addition of sterile saline (0.9% NaCl) based on the optical density measurement at 530 nm.

### 2.5.3 Antibacterial activity

Antibacterial activity of the plant extracts was determined by the disc diffusion method [14]. As media MHB was used. Into sterile petri dish 15 ml of molten media was taken to make plates. The plates were allowed to solidify for 5 minutes. Using the micropipette, 100  $\mu$ l inoculum suspensions that were previously standardized were swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The crude extract at a concentration of 0.1 g/ml was dissolved in 100% dimethyl sulfoxide (DMSO) followed by sterilization using a 0.2 mm Millipore disposable filter. Whatmann filter paper (No.1) discs of 6 mm diameter were made by punching the paper. The extracts at concentration of 500  $\mu$ g/ml were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of the medium and the compound was allowed to diffuse for 5 minutes and the plates were kept in incubation at 37°C

for 24 hrs. Kanamycin was used as positive control at the dose of 30 µg/disc. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. DMSO added disc was taken as negative control to determine the possible inhibitory activity of the diluent of the extract. The zone of inhibition (ZI) was measured in millimeters.

### 2.5.4 Antifungal activity

The disc diffusion method was used for the determination of antifungal activity of the plant extracts [14]. SDB was used as a media. The plates were made by discharging 15 ml molten media into sterile petri dish. Then the plates were permitted to harden for 5 minutes. Using the micropipette, 100 µl inoculum suspensions (previously standardized) were rubbed homogeneously and the inoculum was permitted to dry for 5 minutes. The crude extract with a concentration of 0.1 g/ml was dissolved in 100% DMSO and sterilized by filtration using a Millipore disposable filter having 0.2 mm diameter in size. Whatmann filter paper (No.1) discs having 6 mm in diameter were prepared by punching the paper. The extracts at concentration of 500 µg/ml were occupied on 6 mm sterile disc. The filled disc was positioned on the surface of the medium and the compound was permitted to diffuse for 5 minutes and the plates were reserved for incubation at 25°C for 72 hrs.

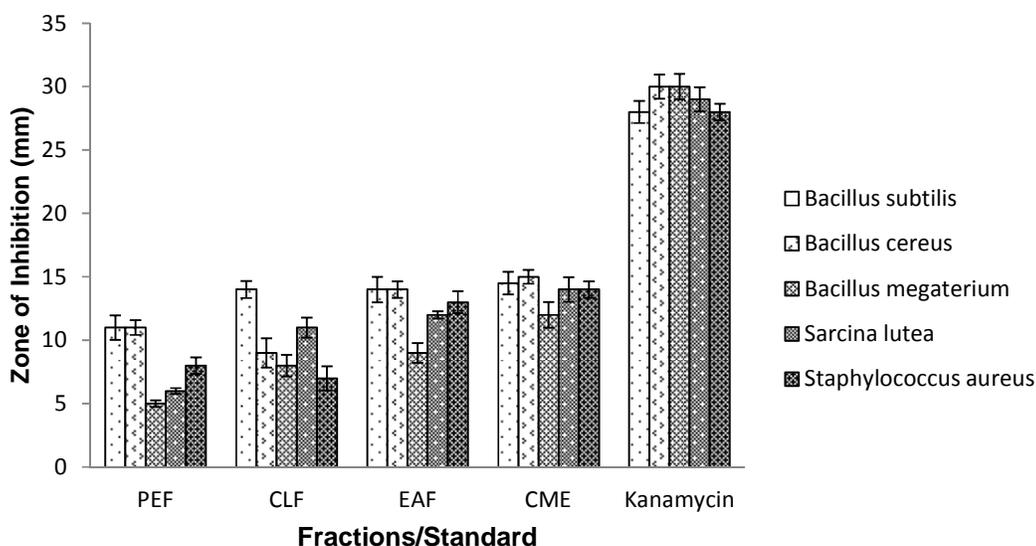
Griseofulvin at the dose of 30 µg/disc was used as positive control. After the period of incubation, inhibition zones formed around the disc were measured by using transparent ruler in millimeter. DMSO added disc was considered as negative control to determine the possible inhibitory activity of the diluent of extract. The ZI was measured in millimeters.

## 3. RESULTS

### 3.1 Determination of Antibacterial Effect

The results of antibacterial activity of crude extracts against a number of gram positive and gram negative bacteria are given below in the Fig. 1 and Fig. 2 respectively. The ZI of the plant extract was found to be in the range from 5 to 15 mm against the entire tested microorganisms.

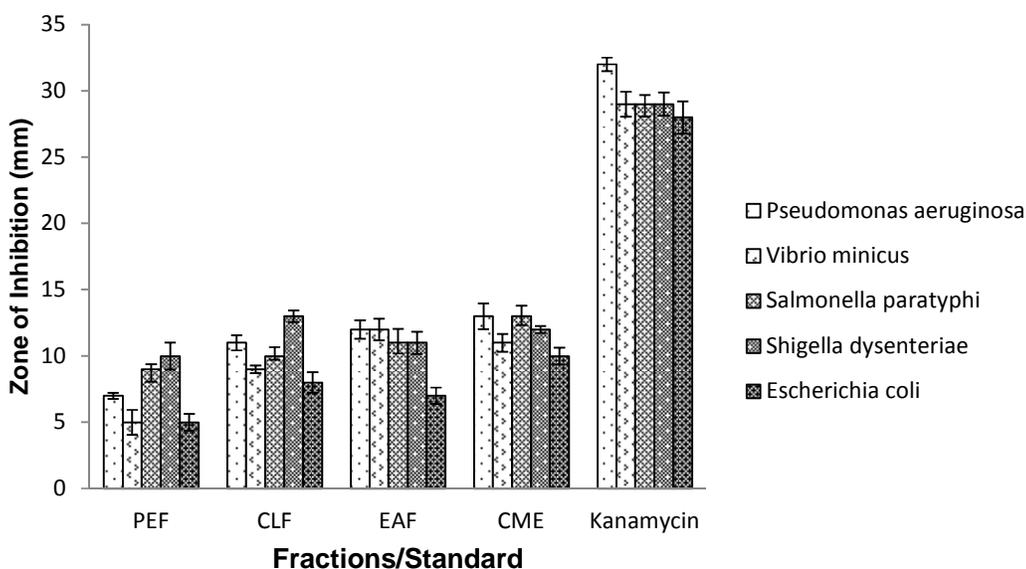
Among the plant fractions CME of leaves of LM showed 15 mm ZI against *Bacillus cereus* and 14 mm against *Sarcina lutea* and *Staphylococcus aureus*. The EAF and CLF of this leaves showed 14 mm ZI against *Bacillus subtilis*. EAF also showed 14 mm ZI against *Bacillus cereus* followed by 11 mm ZI for PEF and 9 mm ZI for CLF. 9 mm ZI was reported against *Bacillus megaterium* for EAF followed by 8 mm for CLF and 5 mm for PEF. EAF showed 12 mm ZI against *Sarcina lutea*, 11 mm ZI for CLF and 6 mm ZI for PEF. Negative control did not show any ZI. Detailed information is listed in Fig. 1.



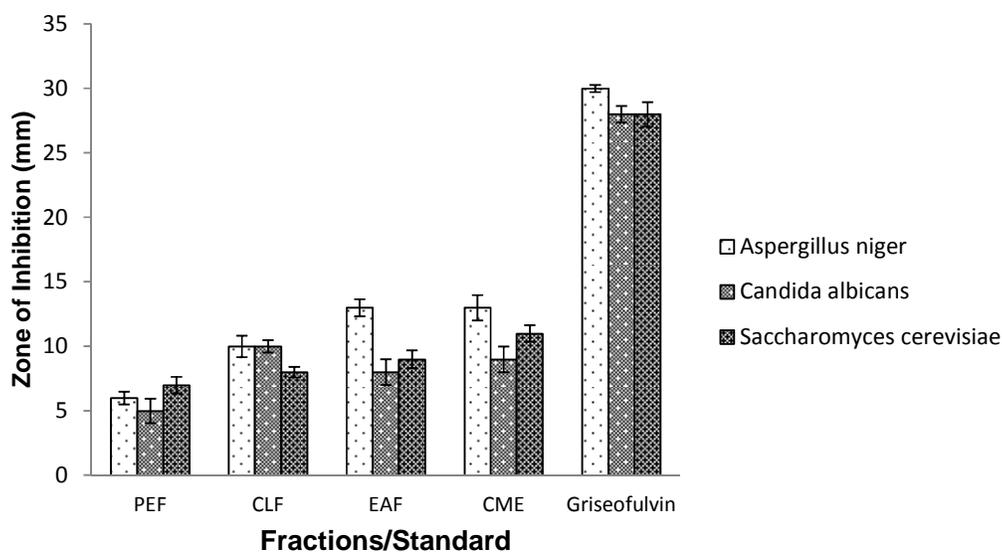
**Fig. 1. Antibacterial activity of LM leaves extracts against gram positive bacteria**  
 Values expressed are mean  $\pm$  SD (n = 3). PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract

The CME of LM leaf showed 13 mm ZI against *Pseudomonas aeruginosa* and *Salmonella paratyphi*. 12 mm ZI was observed by EAF against *Pseudomonas aeruginosa* followed by 11 mm for CLF and 7 mm for PEF. Against *Vibrio minicus* EAF showed 12 mm ZI followed by 9 mm for CLF and only 5 mm for PEF. 11 mm ZI

was observed against *Salmonella paratyphi* for EAF, 10 mm ZI for CLF and 9 mm for PEF. CLF showed 13 mm ZI against *Shigella dysenteriae* followed by 11 mm ZI for EAF and 10 mm ZI for PEF. The minimum ZI was 5 mm for *Escherichia coli* and maximum was 8 mm for CLF given in Fig. 2. Negative control did not show any ZI.



**Fig. 2. Antibacterial activity of LM leaves extracts against gram negative bacteria**  
 Values expressed are mean  $\pm$  SD (n = 3). PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract



**Fig. 3. Antifungal activity of LM leaves extracts**  
 Values expressed are mean  $\pm$  SD (n = 3). PEF = Petroleum ether fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, CME = Crude methanol extract

### 3.2 Determination of Antifungal Effect

The results of antifungal activity of crude extracts of LM leaves are given below in Fig. 3. This study showed that this plant extracts had ZI in the range of 5 to 13 against *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae*. Maximum ZI (13 mm) was recorded against *Aspergillus niger* by CME, then 11 mm against *Saccharomyces cerevisiae* and 9 mm against *Candida albicans*. EAF also showed 13 mm ZI against *Aspergillus niger* followed by 10 mm ZI against *Aspergillus niger* and *Saccharomyces cerevisiae* by CLF followed by 8 mm ZI against *Candida albicans* for EAF and *Saccharomyces cerevisiae* for CLF. EAF had ZI of 9 mm against *Saccharomyces cerevisiae*, 8 mm for CLF and 7 mm for PEF. Negative control did not show any ZI.

### 3.3 Statistical Analysis

Data are expressed as mean  $\pm$  SD from three separate observations. Microsoft Excel 2010 (Roselle, IL, USA) was used for the statistical and graphical evaluations.

## 4. DISCUSSION

In this study the weaknesses of the test organisms to plant extracts was reported. Among 5 gram positive bacteria strongest to moderate activity (i.e., ZI) was reported for CME against *Bacillus cereus* (15 mm), *Bacillus subtilis* (14.5 mm), *Sarcina lutea* (14 mm), *Staphylococcus aureus* (14 mm), EAF against *Bacillus subtilis* (14 mm), *Bacillus cereus* (14 mm), *Staphylococcus aureus* (13 mm), *Sarcina lutea* (12 mm) and CLF against *Bacillus subtilis* (14 mm), *Sarcina lutea* (11 mm) followed by PEF against *Bacillus subtilis* (11 mm), *Bacillus cereus* (11 mm). However, weak activity was reported by EAF against *Bacillus megaterium* (9 mm), CLF against *Bacillus cereus* (9 mm), *Bacillus megaterium* (8 mm), *Staphylococcus aureus* (7 mm) and PEF against *Staphylococcus aureus* (8 mm), *Sarcina lutea* (6 mm), *Bacillus megaterium* (5 mm). In the study of antibacterial and antifungal activity, Haruna MT. reported that EAF of methanolic extract of *Acalypha wilkesiana* showed 7.5 to 9.5 mm ZI against different strains of *Staphylococcus aureus* [15]. Sekar M, et al. [16] showed that methanolic extracts of *Hibiscus sabdariffa* leaves have 11 mm ZI at a concentration of 1000  $\mu$ g/ml against *Staphylococcus aureus*.

Among gram negative bacteria CME showed strongest to moderate activity against *Pseudomonas aeruginosa* (13 mm), *Salmonella paratyphi* (13 mm), *Shigella dysenteriae* (12 mm), *Vibrio minicus* (11 mm), *Escherichia coli* (11 mm), EAF against *Pseudomonas aeruginosa* (12 mm), *Vibrio minicus* (12 mm), *Salmonella paratyphi* (11 mm), *Shigella dysenteriae* (11 mm) and CLF against *Shigella dysenteriae* (13 mm). Weak antibacterial activity was stated by EAF against *Escherichia coli* (7 mm), CLF against *Pseudomonas aeruginosa* (11 mm), *Salmonella paratyphi* (10 mm), *Vibrio minicus* (9 mm), *Escherichia coli* (8 mm) and PEF against *Shigella dysenteriae* (10 mm), *Salmonella paratyphi* (9 mm), *Pseudomonas aeruginosa* (7 mm), *Vibrio minicus* (5 mm), *Escherichia coli* (5 mm). Afolayan AJ. showed that ethanol extract of *Hydnora africana* has 20 mm ZI against *Pseudomonas aeruginosa*, *Escherichia coli* and 16 mm ZI against *Vibrio cholera* [17].

For the fungi strains, strongest to moderate activity was observed by CME against *Aspergillus niger* (13 mm), *Saccharomyces cerevisiae* (11 mm) and weak activity was identified by CME against *Candida albicans* (9 mm), EAF against *Saccharomyces cerevisiae* (8 mm), CLF against *Aspergillus niger* (10 mm), *Candida albicans* (10 mm), *Saccharomyces cerevisiae* (8 mm), PEF against *Saccharomyces cerevisiae* (7 mm), *Aspergillus niger* (6 mm), *Candida albicans* (5 mm). In the study of ethanolic extract of *Caryota urens* flowers Charles A, et al. [18] reported 20 mm ZI against *Aspergillus niger*. Mousavideh-mourdi k, et al. [19] in the study of *Achillea wilhelmsii* flowers reported that methanolic extract has ZI in the range of 10.33 to 40.66 mm against different strains of *Candida albicans*.

The antimicrobial activities of the plants extract is due to the presence of various phytoconstituents such as phenolics, terpenoids, essential oils, alkaloids, lectins and polypeptides, polyacetylenes etc [20]. The antibacterial activity of CME and EAF of LM showed potent inhibition on the bacteria strains used which includes *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio minicus*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Escherichia coli*. Similar finding were also reported against fungal strains such as *Aspergillus niger* and *Saccharomyces cerevisiae*.

## 5. CONCLUSION

The results of the present study provide an important basis for the use of the CME and EAF extracts from the leaves of LM for the treatment of bacterial and fungal diseases. The crude extract found to be active in this study could also be useful for the development of new antibacterial and antifungal drugs. However, further pharmacological and toxicity studies will be necessary to establish if they could be safely used as antibacterial and antifungal agents.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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