Bioactivity of *Syzygium jambos* methanolic extracts: Antibacterial activity and toxicity

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ABSTRACT

Methanol extracts from *S. jambos* leaves were tested for antimicrobial activity and toxicity. *S. jambos* leaf extract inhibited the growth of 4 of the 14 bacteria tested (29%). Both gram-positive and gram-negative bacterial growths were inhibited by *S. jambos* leaf extract, although gram-positive bacteria appeared more susceptible. Two of the 10 gram-negative bacteria (20%) and 2 of the 4 gram-positive bacteria (50%) tested had their growths inhibited by the extract. The leaf extract also proved to be toxic in the *Artemia franciscana* bioassay, with a 48-h LC_{50} of 387.9 ± 38.8 μg/mL, making it slightly more toxic than Mevinphos (505.3 ± 37.7 μg/mL) and approximately 5-fold less toxic than potassium dichromate (80.4 ± 4.3 μg/mL). Whilst potassium dichromate's LC_{50} remained constant across the 72-hour test period (24-h LC_{50}, 86.3 ± 5.1; 72-h LC_{50}, 77.9 ± 4.9), the extract and Mevinphos LC_{50} values decreased by 72 hours (87.0 ± 11.3 μg/mL and 103.9 ± 12.8 μg/mL, respectively), indicating their similar levels of toxicity in the assay.

Key words: Antibacterial, medicinal plants, methanolic extracts, phytotoxicity, rose apple, *Syzygium jambos*

INTRODUCTION

Plants have long been used as medicines for treating a variety of different diseases and complaints. Phytotherapy in Asia is particularly widespread. Plant preparations and medications continue to be used in the treatment of numerous disorders, including eczema, malaria, respiratory disorders and infectious diseases.[1] For some of these plant treatments, the antimicrobial activity has been proven; however, for many plant-based antiseptics, the evidence is anecdotal or, at best, epidemiological. Many traditionally used antiseptic agents have yet to be subjected to thorough scientific investigation.

Syzygium jambos Alston (syn. *Eugenia jambos* L.; *Jambosa jambos* Millsp.; *Jambosa vulgaris* DC.; *Caryophyllus jambos* Stokes) is an evergreen tree of the family Myrtaceae. It is native to Southeast Asia but has been naturalized in India, especially in the state of Kerala, where it is grown both for its fruit and for its medicinal properties. *S. jambos* has a long history of use in Indian traditional medicine for the treatment of numerous ailments. The fruit has been used as a tonic for the brain and liver and as a diuretic.[2] The flowers are believed to reduce fever, and the seeds were used to treat diarrhea, dysentery and catarrh.[2] In South-American cultures, the seeds have additionally been used as an anesthetic,[2] and recent studies have shown *S. jambos* extracts to have a similar analgesic efficacy to morphine in rats.[3] *S. jambos* leaf decoctions were also used traditionally in the treatment of diabetes,[2] although some studies have shown leaf extracts to be ineffective as antihyperglycemic agents.[4] In Indian traditional medicinal systems, *S. jambos* leaves were also used as a diuretic, an expectorant in the treatment of rheumatism; to treat sore eyes; and as a febrifuge.[2] Bark of the *S. jambos* tree is used to treat asthma, bronchitis and hoarseness.[2] Cuban healers have also used the root to treat epilepsy.[2] *S. jambos* leaf extracts have also been shown to possess antiviral activity towards herpes simplex type 1 and type 2 and towards vesicular somatitis virus.[5,6]

The antiseptic properties of some members of the genus *Syzygium* have been extensively studied. In the commercially most important species *Syzygium aromaticum* (clove), the antiseptic properties are well known. Numerous studies have reported on the antibacterial[7] and antifungal[8] activities of oils and extracts from this plant. Other *Syzygium* species from India (*Syzygium lineare*, *Syzygium cumini* and *Syzygium travancoricum*)[9,10] have also been shown to have antimicrobial activity. Two Australian species have also recently been shown to possess

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antibacterial activity.[11] However, despite its numerous traditional medicinal uses, the antiseptic properties of *S. jambos* remain largely unexamined. Some studies have demonstrated the antibacterial[12] and antifungal[13] activities of *S. jambos* bark extracts. To the best of our knowledge, no studies have examined the antibacterial nature of *S. jambos* leaf extracts. Therefore, the current study reports on the antibacterial properties of *S. jambos* leaf extracts, as well as examining their toxicity, to determine their potential as antibiotic agents.

MATERIALS AND METHODS

Plant material

Collection of plant samples

Syzygium jambos leaves were a gift from Mervyn Cooper of the Queensland Tropical Fruit Association. Leaves were obtained from a single tree, washed in deionized water and processed within 4 hours of collection.

Preparation of crude extracts

Syzygium jambos leaves were dried in a Sunbeam food dehydrator, and the dried material was ground to a coarse powder. One gram of each of the dried plant materials was extracted extensively in 50 mL methanol (Ajax, AR grade) for 24 hours at 4°C with gentle shaking. The extract was filtered through filter paper (Whatman no. 54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant pellet was dissolved in 10 mL 20% methanol. The extract was passed through 0.22-μm filter (Sarstedt) and stored at 4°C.

Antibacterial screening

Test microorganisms

All microbial strains were obtained from Michelle Mendell and Tarita Morais, Griffith University, Australia. Stock cultures of *Aeromonas hydrophilia*, *Alcaligenes faecalis*, *Bacillus cereus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas fluorescens*, *Salmonella newport*, *Serratia marcescens*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were subcultured and maintained in nutrient broth at 4°C.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified Kirby-Bauer[14] disc diffusion method. Briefly, 100 μL of the test bacteria was grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/mL. One hundred microliters of microbial suspension was spread onto nutrient agar plates.

The extract components were tested using 5-mm sterilized filter paper discs. Discs were impregnated with 10 μL of the test sample, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4°C for 2 hours before incubation with the test microbial agents. Plates inoculated with *Alcaligenes faecalis*, *Aeromonas hydrophilia*, *Bacillus cereus*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas fluorescens*, *Serratia marcescens* were incubated at 30°C for 24 hours, and then the diameters of the inhibition zones were measured in millimeters. Plates inoculated with *Escherichia coli*, *Salmonella newport*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were incubated at 37°C for 24 hours, and then the diameters of the inhibition zones were measured. All measurements were to the closest whole millimeter. Each antimicrobial assay was performed in at least triplicate. Mean values are reported in this study. Standard discs of ampicillin (2 μg) and chloramphenicol (10 μg) were obtained from Oxoid Ltd. and served as positive controls for antimicrobial activity. Filter discs impregnated with 10 μL of distilled water were used as a negative control.

Minimum inhibitory concentration determination

The minimum inhibitory concentration (MIC) of the *S. jambos* extract was determined by the disc-diffusion method across a range of doses. The plant extracts were diluted in deionized water across a concentration range of 5 mg/mL to 0.1 mg/mL. Discs were impregnated with 10 μL of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was performed as outlined above, and graphs of the zone of inhibition versus concentration were plotted for each extract. Linear regression was used to calculate the MIC values.

Toxicity screening

Reference toxins for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 1.6 mg/mL solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay. Mevinphos (2-methoxycarbonyl-1-methylvinyl dimethyl phosphate) was obtained from Sigma-Aldrich as a mixture of cis (76.6%) and trans (23.0%) isomers and prepared as a 4 mg/mL stock in distilled water. The stock was serially diluted in artificial seawater for use in the bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using the *Artemia franciscana* nauplii lethality assay developed by Meyer *et al.*[15] for the screening of active plant constituents with the following modifications. *Artemia franciscana* Kellogg cysts were obtained from North American Brine Shrimp, LLC, USA (harvested from the Great Salt Lake, Utah). Synthetic seawater was prepared using Reef Salt, AZOO Co., USA. Seawater solutions at 34 g/L distilled water were prepared prior to use. Two grams of *A. franciscana* cysts were incubated in 1 L synthetic seawater under artificial light at 25°C, 2000 lux with continuous aeration. Hatching commenced within 16-18 hours of incubation. Newly hatched *A. franciscana* (nauplii) were used within 10 hours

of hatching. Nauplii were separated from the shells and remaining cysts and were concentrated to a suitable density by placing an artificial light at one end of their incubation vessel, and the nauplii-rich water closest to the light was removed for biological assays. Four hundred microliters of seawater containing approximately 40 (mean, 39.8; $n = 175$; SD, 19.0) nauplii was added to wells of a 48-well plate and immediately used for bioassay. The plant extracts were diluted to 2 mg/mL in seawater for toxicity testing, resulting in a 1 mg/mL concentration in the bioassay. Four hundred microliters of diluted plant extracts and the reference toxins were transferred to the wells and incubated at 25 ± 1°C under artificial light (1000 lux). A negative control (400 µL seawater) was run in at least triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead was counted. The nauplii were considered dead if no movement of the appendages was observed within 10 seconds. After 72 hours, all nauplii were sacrificed and counted to determine the total number per well. The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis.[16]

RESULTS

Antibacterial activity

One gram of powdered dried *S. jambos* leaves was extensively extracted with methanol and dried under vacuum, resulting in 656 mg of dried extracted material. Resuspension of the dried fraction in 10 mL of 20% methanol resulted in the crude test extract concentration of 65.6 mg/mL. The extract was diluted to a concentration of 15 mg/mL for testing for antimicrobial activity. Ten microliters of extract (150 µg) was tested in the disc-diffusion assay against 14 bacteria [Table 1]. The *S. jambos* leaf extract inhibited the growth of 4 of the 14 bacteria tested (28.6%).

Numbers indicate the mean diameters of inhibition (mm) of triplicate experiments ± standard deviation. '-' indicates no growth-inhibition. Chloramphenicol (10 µg) and ampicillin (2 µg) were used as positive controls.

Both gram-positive and gram-negative bacteria were affected by *S. jambos* leaf extract, although the gram-positive bacteria appeared more susceptible. Of the 10 gram-negative bacteria tested, only *A. faecalis* and *A. hydrophilia* (20%) were inhibited by *S. jambos* leaf extract. The leaf extract also inhibited the growth of 2 (*B. cereus* and *S. aureus*) of the gram-positive bacteria tested (50%).

The relative level of antibacterial activity was evaluated by determining the MIC values for each extract against the bacteria which were shown to be susceptible by disc-diffusion assays. MICs were evaluated in the current studies by disc diffusion across a range of concentrations. This has previously been determined to be a valid method of MIC determination, as MIC values determined by disc diffusion correlate well with those determined by broth-dilution assays.[17] The antibacterial activity was strongest against gram-positive bacteria, especially *B. cereus* (as determined by the minimum inhibitory concentration) [Table 2].

Numbers indicate the mean MIC values of at least triplicate determinations.

Quantification of toxicity

The *S. jambos* leaf extract was diluted to 2000 mg/mL in artificial seawater for toxicity testing, resulting in a 1000-mg/ mL concentration in the *Artemia franciscana* lethality bioassay [Figure 1a]. For comparison, the reference

Table 1: Antibacterial activity of *Syzygium jambos* leaf extract

	Zone of inhibition (mm)		
	***Syzygium jambos* leaf extract**	**Ampicillin**	**Chloramphenicol**
Gram-negative rods			
A. faecalis	12.6 ± 0.5	15.2 ± 1.2	6.3 ± 0.6
A. hydrophilia	9.7 ± 0.8	12.0 ± 1.0	28.7 ± 1.6
C. freundii	-	8.3 ± 0.6	15.7 ± 1.2
E. coli	-	14.7 ± 0.6	17.3 ± 0.6
K. pneumoniae	-	10.3 ± 0.6	21.3 ± 1.5
P. mirabilis	-	17.3 ± 0.6	8.7 ± 0.6
P. fluorescens	-	18.2 ± 0.5	21.2 ± 1.2
S. newport	-	18.7 ± 0.6	20.3 ± 0.6
S. marcescens	-	-	14.7 ± 0.6
S. sonnei	-	14.0 ± 0	14.3 ± 0.6
Gram-positive rods			
B. cereus	10.2 ± 0.5	26.7 ± 0.6	13.3 ± 1.2
Gram-positive cocci			
S. aureus	9.0 ± 0	11.7 ± 2.1	16.0 ± 1.0
S. epidermidis	-	26.3 ± 1.5	12.3 ± 0.6
S. pyogenes	-	17.0 ± 1.0	24.0 ± 1.0

toxins potassium dichromate (800 μg/mL) [Figure 1b] and Mevinphos (2000 μg/mL) [Figure 1c] were also tested in the *Artemia franciscana* lethality bioassay. Both reference toxins were more rapid in their induction of the onset of mortality than the *S. jambos* leaf extract at the concentrations tested. For the reference toxins, the induction of mortality was seen within the first 3 hours of exposure. One hundred percent mortality was evident following 4 hours of exposure. In contrast, a period of 6 hours was required for *S. jambos* extract to induce the onset of mortality, and 24 hours was required to kill 100% of the brine shrimp.

Table 2: Minimum inhibitory concentrations (μg/mL) of *Syzygium jambos* extract against susceptible bacteria

	MIC (μg/mL)			
	A. faecalis	***A. hydrophilia***	***S. aureus***	***B. cereus***
S. jambos	797.5	384.6	346.5	182.6

To determine the effect of toxin concentration on the induction of mortality, the extract was diluted in artificial seawater to test across the concentration range 1000 μg/mL to 15 μg/mL in the *Artemia* nauplii bioassay at 24, 48 and 72 hours [Figure 2]. Table 3 shows the LC_{50} values of *S. jambos* extracts towards *A. franciscana*. No 24-hour LC_{50} values are reported for the *S. jambos* extracts as less than 50% mortality was seen by this time for all concentrations tested. The *S. jambos* leaf extract was slightly more

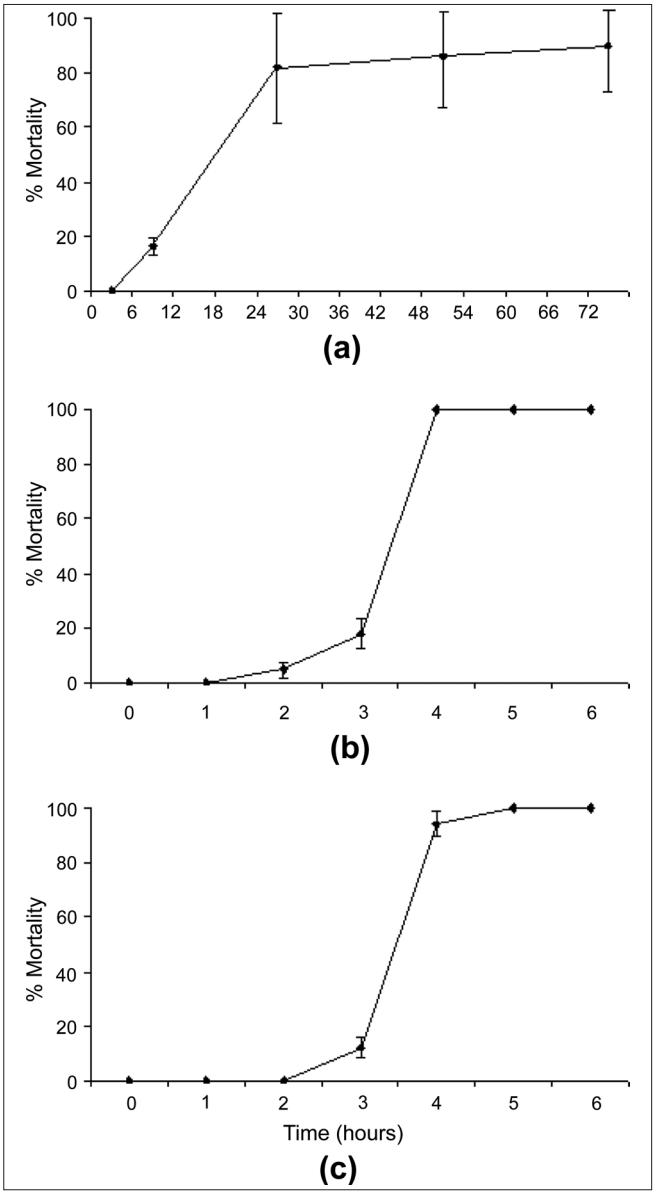

Figure 1: Brine shrimp lethality of (a) *S. jambos* leaf extract (1000 μg/mL), (b) potassium dichromate (800 μg/mL) and (c) Mevinphos (2000 μg/mL). All bioassays were performed in at least triplicate and are expressed as mean ± standard deviation

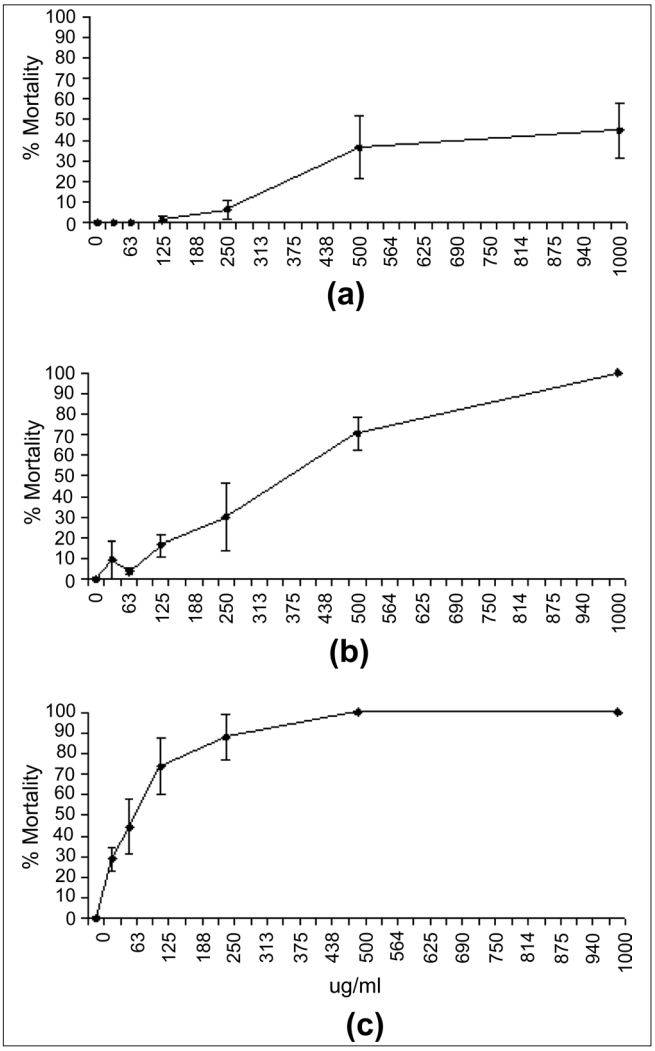

Figure 2: Dependence of *A. franciscana* mortality on *S. jambos* leaf extract concentration following exposure for (a) 24 h, (b) 48 h and (c) 72 h. All bioassays were performed in at least triplicate and are expressed as mean ± standard deviation

Table 3: LC_{50} (95% confidence interval) for brine shrimp nauplii exposed to *S. jambos* leaf extract, the reference toxins potassium dichromate and Mevinphos and a seawater control

	LC_{50}		
	24 h	48 h	72 h
Syzygium jambos leaf extract	-	387.9 ± 38.8	87.0 ± 11.3
Potassium dichromate	86.3 ± 5.1	80.4 ± 4.3	77.9 ± 4.9
Mevinphos	1346.0 ± 78.2	505.3 ± 37.7	103.9 ± 12.8
Seawater control	-	-	-

- denotes values that were not obtained as ≥50% mortality was not obtained at this time point

toxic than Mevinphos at 48 hours, with LC_{50} values of 387.9 ± 38.8 and 505.3 ± 37.7, respectively. Potassium dichromate was substantially more toxic (80.4 ± 4.3) at 48 hours. The extract and both reference toxins showed similar toxicity at 72 hours as seen by their LC_{50} values.

DISCUSSION

The current study reports on the antimicrobial activity and toxicity of *S. jambos* leaf methanolic extracts. The ability of *S. jambos* leaf extract to inhibit the growth of both gram-positive and gram-negative bacteria is in agreement with previous reports of the antibacterial activity of other *Syzygium* species[7-11] and with the reported antibacterial activity of *S. jambos* bark extracts.[12] The greater susceptibility of gram-positive bacteria seen in this study is in agreement with previously reported results for south-american,[18] African[19,20] and Australian[21] plant extracts. Results in this laboratory[11] have also confirmed the greater susceptibility of gram-positive bacteria towards other Australian plant extracts. The gram-negative bacterial cell wall outer membrane is thought to act as a barrier to many substances, including antibiotics.[22] The uptake of the *S. jambos* extract antibiotic agents by gram-negative bacteria is presumably affected by the cell wall outer membrane of some bacteria.

Individual *S. jambos* leaf components responsible for the extract's antiseptic potential were not identified in the current study. However, other reports have identified various bioactive components from *S. jambos*. Scientific studies have demonstrated the anti-inflammatory activity of leaf extracts in rats[23] and have indicated that the flavanoids myricetin and quercetin 3-*O*-b-D-xylopyranosyl (1-2) a-L-rhamnopyranosides are likely to be responsible for this activity.[24,25] Indeed, myricetin and quercetin 3-*O*-b-D-xylopyranosyl (1-2) a-L-rhamnopyranosides isolated during these studies proved to be more effective anti-inflammatory agents than phenylbutazone and indomethacine. *S. jambos* leaves have also been shown to contain 3,3%,4%-tri-*O*-methylellagic acid-4-*O*-b-D-glucopyranoside and 3,3%,4%-tri-*O*-methylellagic acid,[26] as well as several ellagitannins (pedunculagin, casuarinin, tellimagrandin I, strictinin, casuarictin, 2,3-HHDP-glucose and tellimagrandin II),[27] and a number of triterpenes such as friedelin, β-amyrin acetate, betulinic acid and lupeol![13] Future studies need to focus on the purification and identification of the antibacterial and toxic components of *S. jambos* leaf extracts.

The findings reported here also indicate that *S. jambos* extracts display toxicity. This would impact on the usefulness of the extract as a medicinal antiseptic agent. Toxicity was assessed in this study with the test organism *Artemia franciscana*. Whilst toxicity towards *A. franciscana* has previously been shown to correlate well with toxicity towards human cells for some toxins,[28] further studies are required to determine whether this is also true for the *S. jambos* extracts. Toxic antibacterial extracts may be useful as nonmedicinal antibacterial agents (e.g., surface disinfectants). Likewise, toxic plant extracts may also still have medicinal potential even if they are not antimicrobial. McLaughlin *et al*.[28] have demonstrated that toxicity in the *A. franciscana* bioassay may indicate anticancer potential. Toxic extracts such as the *S. jambos* leaf extract should therefore also be tested against human cancer cell lines to determine their potential as anticancer drugs.

CONCLUSION

The results of this study support the traditional use of decoctions of *S. jambos* leaves as antiseptic agents. Furthermore, these findings indicate that *S. jambos* leaf extracts are worthy of further study due to their antibacterial activity. Further evaluation of the antibacterial properties of these extracts against a more extensive panel of microbial agents is warranted. Likewise, purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents. Whilst the extracts examined in this report are promising as antimicrobial agents, caution is needed before these compounds can be applied for medicinal purposes and as food additives to inhibit spoilage. In particular, further toxicity studies using human cell lines are needed to determine the suitability of these extracts for these purposes.

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