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Phytochemicals quantification, tlc and antimicrobial assessment of leaves and fruit extracts of *Lasimorpha senegalensis* (schott) araceae

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ABSTRACT

Aim: This study was aimed to conduct phytochemical screening and evaluate the antimicrobial properties of *Lasimorpha senegalensis*.

Method and Materials: The leaves and fruit parts *of Lasimorpha senegalensis* (Schott) Araceae were collected at Korokorosei community in Bayelsa State, air-dried, pulverized, and extracted using dichloromethane, ethanol, methanol, and water. Flavonoids, saponins, tannins, ketones, and cardiac glycosides were all found to be present. Preliminary thin layer chromatographic screening of the extracts was also done, as well as the antibacterial evaluation of the different fractions using the agar diffusion technique.

Results: The phytochemical screening for tannins, cardiac glycoside, ketones, and flavonoids was positive. Inhibition zones for the aqueous fraction at 62.5, 125, 250, and 500 mg/ml for *E. coli* were 32, 22, 18, and 11 mm respectively, while 8, 9, 10, and 14 mm for *Pseudomonas aeruginosa*. For the methanolic fraction, at the above concentrations, 10, 11, 14 and 14 nm were observed as zones of inhibition on *Escherichia coli*, 14, 19, 15, and 20 nm for *Pseudomonas aeruginosa* and 19, 20, 20, and 20 nm for *Staphylococcus aureus*, respectively. Furthermore, in the dichloromethane fraction, 13, 20, 19, and 15 nm were observed as zones of inhibition on *Escherichia coli*, 8, 10, 15 and 15 nm for *Pseudomonas aeruginosa* and 20, 15, 20, and 18 nm for *Staphylococcus aureus*, respectively.

Conclusion: It was concluded that the leaf portion of the plants can be used in the treatment of infections caused by *E. col, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. With the high amounts of flavonoids observed, it can be employed as an antioxidant and a cardioprotective.

Keywords: Antimicrobial, Araceae, *Lasimorpha senegalensis,* phytochemicals.

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Introduction

Medicinal plants contain chemicals that can be employed for therapeutic purposes or as precursors for manufacturing of effective pharmacotherapeutic drugs in one or more of its components (Da-Cheng, 2019). The synergistic bioactivity of phytochemicals in plant extracts is frequently regarded as a benefit that is difficult to duplicate with single synthesized conventional medications (Abdullahi *et al*., 2020). *Lasimorpha senegalensis* is a monocotyledonous flowering plant with blooms produced on a form of inflorescence spadix, belongs to Araceae family.

A spathe, or leaf-like bract, surrounds and protects the spadix (Bown *et al*., 2000). The plant is known as Swamp arum, with local names like Okwoo-bà (Ijaw/Izon/Korokorosei), Akasi-iyi (Ika), Ede mmiri (Igbo), etc., (Kay Williamson, 2012). *Lasimorpha senegalensis* is a perennial herbaceous plant that grows in marshy places and produces a clump of leaves from a short, thick, stoloniferous rhizome. It is found commonly in Senegal, Sierra Leone, Chad, Nigeria (Bayelsa), Central African Republic of Congo (Vander Burg, 2004).

A short, thick, strongly stoloniferous rhizome creates a clump of leaves for the plant. Each leaf has an erect, spiny petiole up to 100cm long (up to 200cm) and a 50cm long (exceptionally up to 100cm) and 30cm wide arrow-shaped leaf blade (40cm). On a spiny, single peduncle up to 150cm

(250cm) long, emerging from the leaves, the inflorescence is a cylindrical, purplish spadix up to 12cm long, encircled by a spathe up to 45cm long (Protabase, 2014). The plant usually forms large populations due to its strong development of underground suckers. The plant is found /occurs in swampy forests, along streams, in ditches, and ponds and they are often very abundant (Govaerts *et al.,* 2002). The plant is gathered in the wild for usage as food and medicine in the local community. In temperate climates, it might be used as an indoor pot plant, while in warmer climes, it could be used as a garden pond decorative. *Lasimorpha Senegalensis*is highly abundant in nature, especially in swampy environs (DeFilipps & Krupnick, 2018). It is however potentially impacted by agricultural development, invasion by other species (*Cyperus papyrus*), and water pollution. It is classified as least concern in the International Union for Conservation of Nature (IUCN) red list of threatened species (Boos *et al.,* 2003).

Several ethnomedicinal uses of *Lasimorpha Senegalensis* have been reported, including the management of gonorrhea and dysentery. In southern Nigeria, the fruits are reported to be part of the ingredients for many remedies (Anumudu *et al*., 2019). In Sierra Leone, the young leaves are eaten as a famine food and as an ingredient in palaver sauce, while, the young leaves are eaten as vegetables, and the rhizomes are used to treat ulcers in Gabon (Adamu *et al*., 2005). Also, in Congo, the leaves are taken to cure cough and in larger doses to treat nervousness and agitation. It is also given to women during childbirth to accelerate delivery (Adamu *et al*., 2005). Furthermore, the leaf sap has been taken orally against hiccups in Côte d'Ivoire and the Eastern part of Nigeria (Igbos). Hepatitis and feverish diseases have also been treated with it. (Vander Burg, 2004; Dalziel *et al.,* 1937). *Lasimorpha senegalensis* has been reported to contain calcium oxalate crystals which are toxic if consumed raw. However, the calcium oxalate can easily be broken down by thoroughly cooking the plant or by fully drying it. Moreover, caution should be taken when including this plant in the diet of people suffering from rheumatism, arthritis, gout, kidney stones, and hyperacidity as it could induce adverse side effects which can lead to death (Vander Burg, 2004; Dalziel *et al.,* 1937).

There are previous reports on *Lasimorpha senegalensis,* Araceae, regarding its geographical distribution, edible and medicinal uses. Various Araceae species are used to cure malaria and its symptoms throughout the world's tropical regions (Frausin *et al.,* 2015). Antimalarial species belonging to the genus *Amorphophallus Blume*, *Culcasia, Homalomena*, and others have been discovered in the African countries of Ivory Coast, Kenya, Gabon, Benin, and Togo, according to reports. The biggest number of Araceae plants are utilized as antimalarials in the Amazon region (Pedralli, 2002; Frausin *et al*., 2015). The Amerindian ethnic groups employ the Neotropical genera; *Philodendron* Schott and *Anthurium* Schott: *Yanomami* (Brazil), *Tirios, Waypi* (French), *Makuna*, and *Miraa* (Colombia), as well as *Secoya* and *Tacana* (Pacific Coast, Colombia) (Frausin *et al.,* 2015). Surprisingly, no antimalarial Araceae genus was employed on both the African and American continents, indicating that the species used had a regional range (Ayoola, 2008). The decoction was the method of extraction most cited for antimalarial remedies for species of Araceae. Leaves and the tubercles were the parts most often cited (Ayoola, 2008). Another study examined *L. senegalensis* (Schott) leaf extract's antioxidant and hepatoprotective properties. The findings revealed that the leaf extract contained significant levels of bioactive phytochemicals as well as free radical scavenging activities. The extract also increased endogenous antioxidants and lowered lipid peroxidase and liver enzymes considerably (Chinyere *et al.,* 2020).

Traditional medicine, despite being an old practice in illness prevention and treatment, is still widely used around the world to treat a variety of human ailments. Methanolic and aqueous extracts of *L. senegalensis* were tested for antibacterial activity against human pathogens, *Escherichia coli*, and *Staphylococcus aureus*, in a study conducted between 2018 and 2019. (Anumudu *et al.,* 2019). The efficacy of *L. senegalensis* against the test organisms at various concentrations was determined using the agar well diffusion method.

Tetrazolium chloride microtiter dilution experiment was used to establish the minimum inhibitory concentration (MIC). The inhibitory zone widths for both test organisms employing plant extracts ranged from 0 to 14 mm, which was less than the control (Trimethoprim / Sulfamethoxazole and chloramphenicol) which ranged from 0 to 26 mm. The MIC was 62.5 mg/ml to 500 mg/ml. Methanolic stem extract yielded the lowest MIC

(Karunanidhi *et al.,* 2013).

Preliminary phytochemical screening revealed the presence of flavonoids responsible for antibacterial activity. As a result, *L. senegalensis* is deemed medicinally important because it contains physiologically active chemicals with anti-infectious disease potential (Anumudu *et al*., 2019). Hence, the current study was aimed to conduct phytochemical screening of the leaves and fruits of *Lasimorpha senegalensis* (Schott) using different solvents, determine the possible fractions present by thin-layer chromatography, and screen for its potential antimicrobial properties using clinical isolates of *Neisseria gonorrhea, E. coli, S. aureus, amoeba histolitica* and *candida albican*.

Materials and Methods

Reagents

Ethanol, methanol, distilled water, dichloromethane, 10% ammonia, concentrated sulfuric acid, resorcinol, concentrated hydrochloric acid, ferric chloride, acetic acid, n-Hexane, 5% ferric chloride, normal saline, distilled water, Amoxicillin (25ug) disc.

Location of Plant Collection

The leaves and fruits of *Lasimorpha senegalensis* (Schott), Araceae, were collected from the wild, after obtaining ethical approval from the Community Development Committee, at Korokorosei kingdom; latitude: 4.750752, [4°45'02.7"N], longitude: 6.006715 [6°00'24.2"E], Olodiama clan, in Southern Ijaw Local Government Area, Bayelsa State, Nigeria. The Korokorosei Kingdom borders other communities including Ikienghebiri, Ikebiri I, Olugbobiri, Ondewari, Okpotuwari, Azuzuama, and other settlements, all surrounded by aquatic environs. The plant was authenticated by Prof. Alade O. Gideon, at the Pharmacognosy and Herbal Medicine Department, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, with herbarium number: PCG-UG-15-3321.

Plant Extraction

The leaves and fruits of *Lasimorpha senegalensis* (Schott) Araceae were collected, air-dried, and pulverized to a coarse powder using a mechanical blender. About 903g of the coarse leaf powder was transferred into a Winchester bottle and cold-macerated for five days with about 3.8L of methanol. About 801 g of the coarse fruit powder was also transferred into Winchester bottles and 3.8 L of methanol was used to extract using the cold maceration technique for a week. The powder was filtered and the filtrate was concentrated using a water bath at 44°C. The concentrated extracts were allowed to cool and they were stored safely.

Phytochemical Screening

Flavonoids, Saponins, Tannins, Ketones, and Steroids (Cardiac glycosides) were screened for phytochemicals and secondary metabolites using conventional techniques (Auwal et al., 2014; Nwankwo *et al.,* 2021).

Saponins can be detected using the following test: In a test tube, 0.5g of the plant extract was added to 10ml of distilled water and briskly shaken. After strong shaking, no foaming forms, indicating the absence of Saponins in the leaf and fruit.

For Cardiac glycoside, 0.5g of extract was dissolved in 5ml chloroform and then filtered. In the test tube, 1 mL of acetic acid was added. To build a layer underneath, 1ml concentrated sulfuric acid was slowly introduced through the test tube's side. The presence of the steroidal nucleus is shown by a shift in color from violet to green in the leaf and fruit extracts.

For flavonoids, 5 mL of 10% ammonia solution was added to a part of the extract's aqueous filtrate, followed by a concentration and equal volume of conc. sulfuric acid. The presence of flavonoids in the leaf and fruit is indicated by the yellow hue.

For Tannins: 10ml distilled water was added to the extract. After which, a couple of drops of ferric chloride were added. The presence of tannins in the leaf and fruit is indicated by the brownish-green coloring. 2ml conc. HCl plus a few drops of resorcinol. The presence of ketones in the plant's leaf and fruit is indicated by the rose coloration. Three factions were prepared for the leaf including aqueous, methanol, and dichloromethane fractions, while aqueous, ethanol, and dichloromethane fractions were prepared for the fruit.

Thin Layer Chromatography (TLC)

The different fractions were spotted using a capillary tube on the plate. A saturated chamber containing the solvent system n-hexane and Ethyl acetate in a ratio of 5:2, and 9:1, respectively were developed. The TLC plate was then transferred into the chamber. After 15 minutes, the plate was removed from the chamber when the solvent had gotten to the solvent front. The plate was allowed to dry and then viewed under an ultra Violet Lamp and the spots seen were noted with a sharp pencil. It was stained with anisaldehyde and sulfuric acid and allowed to dry, after which the spots became

visible. The distance moved by the solute and the distance moved by the solvent front was noted and the retardation factor was calculated.

Antimicrobial Screening

Preparation of Stock solution and serious dilution**:** 0.5g of the extract in 0.5ml of 0.5% DMSO was shaken properly to dissolve the extract. Then 4.5ml of distilled water was added to the solution. Five concentrations (500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml) were prepared for each fractions (Aqueous fraction, DCM fraction, Methanol fraction). And for the fruit, five concentrations were prepared using the different fractions. The antimicrobial screening was carried out against the following organisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Protis*. All these organisms were collected from the laboratory stock stored in the refrigerator. The test organisms were cultured from the stored of a molten agar plate and incubated at 37°C for 24hours. The various bacteria culture or isolates were standardized using a sterile bottle containing 5ml of sterile water and the turbidity was adjusted and compared to McFarland's standard, (A 0.5ml of McFarland's standard was prepared by mixing 0.05ml of 1.175% barium chloride dehydrate ($BaCL_2.2H_2O$) with 9.95ml of 1% sulfuric acid (H_2SO_4).

Agar Preparation and Susceptibility Testing for Anti-microbial

The weight of the agar-agar was calculated based on the amount in milliliters (40ml) of agar needed and this was transferred into a beaker containing distilled water and autoclaved at 121°C for 15minutes. The agar was then poured into the Petri dishes allowed to cool and set and transferred into a hot air oven for final drying. These processes were carried out under aseptic conditions to avoid contamination. The standardized bacteria suspension was poured into the Muller Hinton Agar plates and the excess fluid was poured into a beaker containing sodium hypochlorite. In other to ensure a uniform and confluent growth, the suspension was poured twice over the entire surface by repeating the procedure, taking care the second time to turn the plate through 60°. The inoculum on the agar plate was allowed to dry for 5-15minutes. A sterile cork-borer was used to prepare four holes of 10milliliter in diameter, in each agar plate aseptically at a distance of 15mm apart. Using a sterile pasture pipette, two drops of molten agar were used to seal each hole. The concentration of 500mg/ml, 250mg/ml, 125mg/ml, and 62.5mg/ml of the test sample extract was introduced into the various hole respectively and allowed to stand for 1hour for sufficient pre diffusion of the extracts to occur. This method was used to test each fraction (Aqueous fraction, methanol fraction, and dichloromethane fraction) against each of the test organisms (*Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris*) separately and incubated at 37°c for 24 hrs and inhibition zone measured and recorded appropriately.

Results and Discussion

The extraction of the leaf with methanol yielded 2.2%w/w and the extraction of fruit with ethanol yielded 1.9%w/w. The results of the phytochemical screening for tannins, cardiac glycoside, ketones, and flavonoids were positive. While phytochemical screening for saponins was negative for the leaves and fruits of *Lasimorpha senegalensis* (Fig 1 and Table 1).

Fig. 1. Leaves (aerial parts) and fruit of Lasimorpha senegalensis

Table 1: Phytochemical and Secondary Metabolites screening

Phyto-chemicals	Positive indicator	Results	
		Leaf	Fruit
Saponins	Frothing which persists in warming	-	--
Flavonoids	A yellow coloration	++	+++
Cardiac glycoside	A change in color from violet to green.	+++	+
Ketones	A rose coloration	+++	+++
Tannins	A brownish-green	++	+

Key: +++ = densely present, + = present, -- = absent

Table 2: TLC Analysis

Sample	Solvent System (n-Hex: ETA)	Spots	Rf value	Solvent System (n-Hex: ETA)	Spots	Rf value
Leaf (Methanol)	9:1	1	0.8	5:2	1	0.1
	9:1	2	0.2	5:2	2	0.2
	9:1	3	0.3	5:2	3	0.4
	9:1	4	0.4	5:2	4	0.6
	9:1	5	0.6	5:2	5	0.6
	9:1	6	0.7	5:2	6	0.8
	9:1			5:2	7	0.9
Fruit (Methanol)	9:1	1	0.1	5:2	1	0.3
	9:1	2	0.8	5:2	2	0.8
Leaf (DCM)	9:1	1	0.59	5:2	1	0.1
	9:1	2	0.7	5:2	2	0.2
	9:1	3	0.8	5:2	3	0.4
	9:1	4	0.9	5:2	4	0.6
	9:1	0		5:2	5	0.7
	9:1	0		5:2	6	0.8
	9:1	0		5:2	7	0.9
Fruit (DCM)	9:1	1	0.9	5:2	1	0.9
Leaf (Aq)	9:1	1	0.8	5:2	0	
Fruit (Aq)	9:1	0		5:2	0	

Key: *Aq = Aqueous, n-Hex = n-hexane, ETA = Ethyl acetate, Rf = Retention factor*

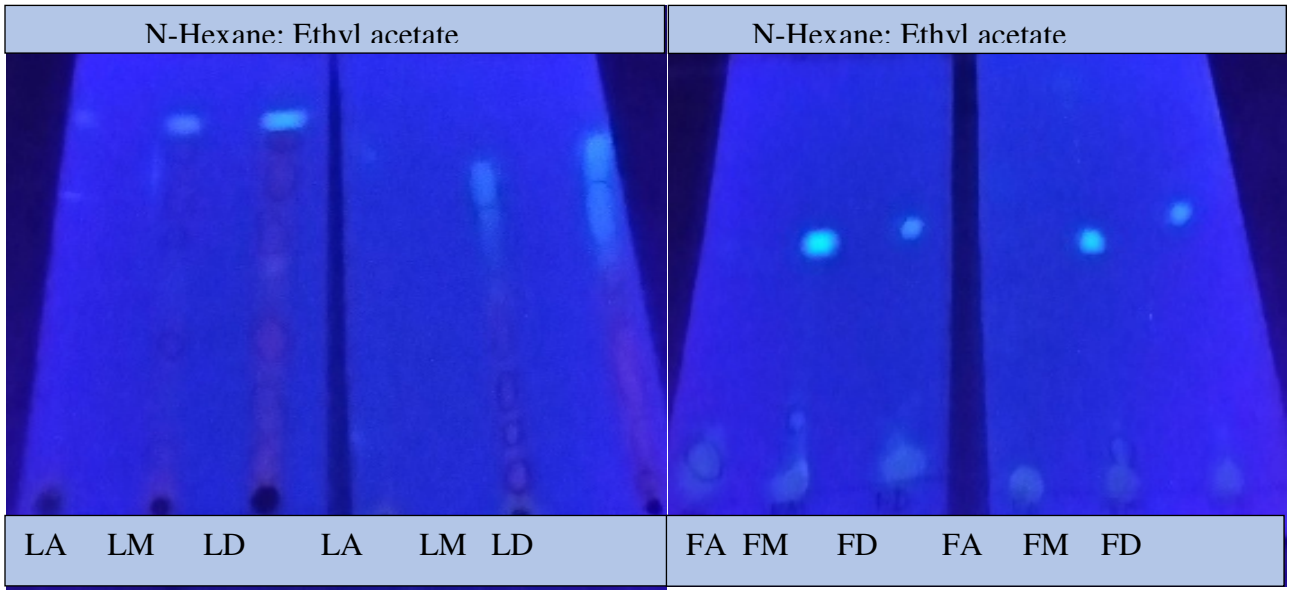

Fig 2: TLC plates of the leaf and fruit extract under UV lamb: (L – Leaf, F – Fruit, A – Aqueous, M – Methanol, D – Dichloromethane)

Table 3: Showing antimicrobial activity of the leaf Lasimorphasenegalensis(Schott) Araceae

Organisms	Aqueous fraction (mg/ml)				Methanol fraction (mg/ml)				Dichloromethane fraction (mg/ml)				(H_2O)	Amoxicillin
Conc (mg/ml)	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5	(0.3ml)	(25µ)
E. coli (mm)	11	18	22	32	14	14	11	10	15	19	20	13	0	37
Pseudomonas aeruginosa(mm)	14	10	9	8	20	15	19	14	15	15	10	8	0	27
Staphylococcus aureus(mm)	0	0	0	0	20	20	20	19	18	20	15	20	0	28
Proteus vulgaris (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	10

Key: *mm = millimeter, mg/ml – milligram per milliliter,µ - microgram*

The TLC showed that the leaf aqueous fraction with the solvent ratio of n-hexane and ethyl acetate (5:2) showed the presence of one (1) spot, and the dichloromethane fraction showed seven (7) spots, and the methanol fraction showed six (6) spots (Table 2). This specifies that the plants have several chemical constituents with each spot indicating a particular chemical entity as was obtained from the retention factors values. For the second solvent ratio of n-hexane and ethyl acetate (9:1), the aqueous fraction showed one (1) spot, seven (7) spots for dichloromethane, and six (6) spots for the methanol fraction, depicting the presence of several chemical constituents (Figure 2). The antimicrobial susceptibility test conducted using the leaf extracts showed some activities against *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* except aqueous fraction that did not show any antimicrobial activity against *Staphylococcus aureus* and *Proteus vulgaris*. All the fractions showed no activity against *Proteus vulgaris*. Compared with the standard drug, (Amoxicillin), the zones of inhibition for the aqueous fraction at 62.5, 125, 250 and 500 mg/ml for *E. coli* were 32, 22, 18, and 11 mm respectively, while 8, 9, 10 and 14 mm for *Pseudomonas aeruginosa* at the same concentration. No inhibition was observed for *Staphylococcus aureus* and *Proteus vulgaris.* A dose-dependent response was observed from the above activities, for the E. coli, as the dose was decreased, a higher zone of inhibition (antimicrobial activity) was obtained, while the reverse was seen in the case of *Pseudomonas aeruginosa* (Table 3).

For the methanolic fraction, at the above concentrations, 10, 11, 14, and 14 nm were observed as zones of inhibition on *Escherichia coli*, 14, 19, 15, and 20 nm for *Pseudomonas aeruginosa*, and 19, 20, 20, and 20 nm for *Staphylococcus aureus*, respectively. Thus, more activities were seen against *Staphylococcus aureus* (a gram-positive organism) and *Pseudomonas aeruginosa* (a gram-negative organism). Furthermore, in the dichloromethane fraction, 13, 20, 19, and 15 nm were observed as zones of inhibition on *Escherichia coli*, 8, 10, 15, and 15 nm for *Pseudomonas aeruginosa*, and 20, 15, 20, and 18 nm for *Staphylococcus aureus*, respectively (Table 3). These findings are similar to previous findings on the antimicrobial properties evaluation *of Lasimorpha senegalensis*, (Anumudu *et al.,* 2019). The leaf section of the plants can also be utilized to treat disorders or infections caused by *E. coli*, urinary tract infections, gastroenteritis, neonatal meningitis, hemorrhagic colitis, and Crohn's disease, according to the findings (Todar K., 2007); *Pseudomonas aeruginosa*, such asbronchopneumonia, septic shock, urinary tract infection, GIT infection, skin and soft tissue infections (Todar, 2004); and *Staphylococcus aureus* (bacteremia, urinary tract infections, abscess (boils), cellulitis, meningitis, soft tissue infections, pneumonia, and septicemia, as well as food poisoning (Kuehnert *et al.,* 2005; Tong *et al.,* 2015 Woodson, 2017).

Conclusion

The results obtained from the antimicrobial susceptibility test showed activities against *Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*, but no efficacy on *Proteus vulgaris*. Thus, it is a potential antimicrobial plant that can be explored in the development of use antibacterial agents. The results obtained show that the plant leaves when standardized can be used as a supplement for heart ailments as it contains a high level of cardiotonic steroids. The plant contains a high amount of flavonoids, hence, it can be employed as an antioxidant, cardioprotective, etc. The leaf also showed the presence of tannins which have been reported to be present in most medicinal formulations used in the management of sexual dysfunction, diabetes, and urinary and respiratory tract infections. Therefore, further is required for its standardization and isolation of active constituents.

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