

Green Synthesis of Silver Nanoparticles with Combinatorial Leaf Extracts: Phytochemical Screening and Antibacterial Activity

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ABSTRACT

The Pharmacological properties of medicinal plants aid in the treatment of various diseases are emerging area of research in the present scenario due to the presence of secondary metabolites. The present study aims to explore the green synthesis of silver nanoparticles from leaf extracts and the evaluation of bioactive compounds and its antibacterial activity. The present research focused on the analysis of the phytochemical composition, antibacterial activity, and green synthesis of silver nanoparticles from the leaf extracts of *Cassia fistula*, *Adhatoda vasica*, and *Aegle marmelos*. Following aqueous extraction, phytochemical screening and antibacterial activity were carried out. Green synthesised silver nanoparticles were characterized by using UV absorption, FTIR, and X-Ray Diffraction methods, and its antibacterial activity was evaluated. The hot aqueous combinatorial extract was quantitatively analyzed for the presence of tannins (3.03 mg), flavonoids (2.4 mg), alkaloids (10.4 mg), phenols (10.2 mg), and carbohydrates (7.8 mg). The green synthesis of silver nanoparticles was indicated with a UV-visible absorption peak at 460 nm. Functional groups identified by FTIR include amines, nitro compounds, alcohols, phenols, and alkenes. The highest size of synthesised silver nanoparticles from the combinatorial leaf extract was 23.63 nm (200) using XRD. Antibacterial activity of green synthesised silver nanoparticles demonstrated 13 mm zone of inhibition for *Staphylococcus sp.* and a zone of 15 mm for *Escherichia coli*. This present investigation paves a path for the establishment of green synthesised combinatorial leaf extracts that exhibit greater antibacterial efficacy against various pathogens. Hence, novel drug formulations may be adapted in the future.

KEY WORDS: ADHATODA VASICA, ANTIBACTERIAL ACTIVITY, CASSIA FISTULA, GREEN SYNTHESIS, PHYTOCHEMICAL ANALYSIS, SILVER NANOPARTICLES.,

INTRODUCTION

The primary source of natural products that are widely and successfully used in medicine is plants. Disease incidence is typically lower in populations that use a lot of natural herbal items. Recently, there has been a lot of attraction towards natural-based herbs as antimicrobial agents due to their eco-friendly and health hazardless in nature. The traditional Indian systems of Ayurveda and Siddha medicines support

the importance of medicinal plants to treat diseases Ameer et al., (2021). Herbal medicines have been utilized in most countries since ancient times. Still medicinal plants in Asia are extensively employed as a therapy for infectious disease in rural and background areas, Omeje et al., (2023).

Herbal medications are commonly used in healthcare due to their inexpensive cost and abundance of antibacterial qualities (Enechi et al 2022). Many plants and herbal medicine-derived natural products could be used as an alternative therapeutic potential for RTI since they have antibacterial effects, Armutcu et al.,(2021). The size of nanocarriers was similar to biological molecules like

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viruses and proteins, which allows them to interact with cell surfaces and the cell wall Lombardo et al. (2019). Silver nanoparticles were used in a broad range of applications like drug delivery, food industries, anti-microbial and anti-cancer studies on the green synthesis of silver nanoparticles Zharkova et al. (2021). Therefore, plant extract-mediated green synthesis of nanoparticles is considered eco-friendly, cost-effective, and safe, and is a viable alternative for microbiological applications Garibo et al. (2020).

MATERIAL AND METHODS

The Following plants were used in the present study.

Cassia fistula: A deciduous tree with lovely yellow blossoms and grey bark is *Cassia fistula*. In Ayurvedic and Unani medicine, it is one of the most often utilized plants. According to some reports, this plant can help with skin conditions, liver issues, tuberculous glands, and diabetes. The fruit pod measures 20–27 mm in diameter and 40–70 cm in length. The distal extremities of the fruit pod are rounded and somewhat bent. Danish et al.(2011). This medium-sized, ornamental plant grows quickly and sheds its leaves after the growth season. Hanif et al.,(2007). Numerous quantities of primary and secondary metabolites are present in *Cassia fistula*. The pharmacological and biological effects of this substance depend on these metabolites. Anthraquinones, polyphenols, polysaccharides, flavonoids, tannins, glycosides, and amino acids are examples of primary and secondary metabolites.

Adhatoda vasica: For over two millennia, *Adhatoda vasica* has been utilized in India's traditional medical system. It is also a well-known medication in Unani and Ayurvedic medicine. The antibacterial, anti-spasmodic, anti-arthritic, antiseptic, expectorant, anti-tuberculosis, and anti-cancer qualities of *Adhatoda vasica* are well-known.

Aegle marmelos: There are various uses for *Aegle marmelos* as a medicinal plant. Every portion of *Aegle marmelos*, including the leaves, fruits, pulp, flowers, stem bark, and root bark, had therapeutic value; however, the leaf had the strongest pharmacological activity. Leaves were employed as a mild laxative or to relieve asthmatic mucous membrane inflammation. Leaf decoction can be used as an expectorant, to help get rid of fever, or to help clear the bronchial passages of mucus discharge. *Aegle marmelos* leaves can treat severe conjunctival inflammation, including acute bronchitis, and they can also treat inflammation in other parts of the body.

The leaves of *Cassia fistula*, *Adhatoda vasica*, and *Aegle marmelos* were gathered from the Tirupur district in Tamil Nadu, India. Plants were identified and authenticated by Botanical Survey of India vide certificate no BSI/SRC/5/23/2024/Tech/892. After the collection plants being cleaned with water, the recently harvested leaves were promptly drenched with ethanol and allowed to dry at room temperature in the shade. In a mixer, the dried leaves were ground into a powder. For later use, the powdered plant leaf material was kept in sterile glass containers.

Extraction of Plant Materials: All the dried leaf powders were combined into various concentrations such as 1:1:1, 0.5:0.5:1, 0.5:1:0.5, 1:0.5:0.5 and 0.5:0.5:0.5. The manufacture of the extract was done using these blended concentration granules. The combination was steeped for 24 hours after 10 grams of each concentration powder were suspended in 100 ml of hot and cold distilled water. The clear solution was stored in a water bath at 80°C for two hours after the residues were filtered through Whatman No. 1 filter paper. For later usage, the dehydrated crude extracts were kept at 4°C (Chessbrough (2000).

Collection of Clinical Pathogens: The Government Hospital in Erode was where the clinical pathogens were collected. For the following investigation, isolates were kept on nutrient agar at 4°C. The conventional Gram-staining, biochemical, and selective plate methods were used to confirm the clinical pathogens. After streaking the stains on selective media, they were incubated for 24 hours at 37°C. *Escherichia coli* streaked on an EMB agar plate and *Staphylococcus sp.* on an MSA plate.

Antibacterial Activity of Combinatorial Leaf Extract: The usual agar well diffusion method was utilized to test the plant extract's antibacterial effectiveness against clinical pathogen microorganisms. After swabbing the cultures onto the nutrition agar plates, wells of 6 mm in diameter were created on the nutrient agar using gel pierce, and 100 µl of plant extract was added to the wells created using the gel borer. following a 24-hour incubation period at 37°C. Syeda and Riazunnisa (2020).

Qualitative Phytochemical Analysis: Using routine protocols, a preliminary screening for phytochemicals was conducted. Audu, Mohammed and Kaita,(2007). To analyze the phytoconstituents found in plants, tests for alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, glycosides, and proteins were conducted.

Quantitative Phytochemical Analysis: Alkaloids, flavonoids, carbohydrates, tannins, and phenols were all quantitatively analyzed using conventional techniques. Anandhu et al.,(2021).

Hemolytic Activity Of Combinatorial Leaf Extract: With a little modification of the Yang, Sun and Fang , (2005) approach, the cytotoxicity of plant extract to normal, healthy cells was examined using in vitro hemolytic activity. The plant extracts were streaked over a blood agar plate, then incubated for 48 hours at 37°C. Extracts that did not produce hemolysis were chosen for additional processing after incubation.

Green Synthesis of Silver Nanoparticles Using Combinatorial Leaf Extract: After being soaked for the whole night, 15g/l of plant powder was extracted in a water bath at 60–70 °C for 20–30 minutes and then chilled. Next, two ml of 0.1 M AgNO₃ solution were added to eight ml of extract. It was heated in a water bath for 40 minutes at a temperature of 60°C. After 30 minutes, the reaction mix's color changed, but it was still incubated for 24 hours at room temperature to improve synthesis. Following incubation,

the mixture was centrifuged for 20 minutes at 5000 rpm to confirm the formation of nanoparticles by UV-visible spectroscopy. For additional characterization research, the resulting pellets were gathered, suspended three times in distilled water, then centrifuged to eliminate any remaining unbound biomass. The nanoparticles of silver were kept in storage.

Characterization of Silver Nanoparticles Using Combinatorial Leaf Extract UV Absorption Spectrophotometric Analysis of Green Synthesized Silver Nanoparticles: The UV absorption spectrophotometer was used to analyze the produced silver nanoparticles. About

1 mg of dried nanoparticles and 9 ml of ethanol were combined to prepare the sample, which had an absorption wavelength in the 350–600 nm. Iman et al.(2023).

Fourier Transform Infrared Spectrophotometric Analysis of Green Synthesized Silver Nanoparticles: The functional compounds that comprise green produced silver nanoparticles are identified by FTIR analysis. A sample for examination was made by mixing 0.05 mg of nanoparticles with KCl. After that, the sample was put into an FTIR device, and the spectrum was captured. With a resolution of 4 cm⁻¹, spectra were captured between 400 and 4000 cm⁻¹. Wilson and Venkateshwari (2022).

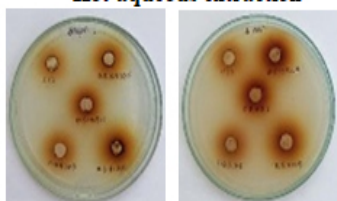
Table 1. Qualitative phytochemical analysis

PHYTO CHEMICALS	TEST	RESULT		
		<i>C. fistula</i>	<i>A. vasica</i>	<i>A. marmelos</i>
Alkaloid	Wangner'stest	+	+	+
lavonoid	Alkaline reagent test	+	+	+
Saponin	Foam test	-	+	-
Tannin	Ferric chloride test	-	-	+
Protein	Ninhydrin test	+	+	+
Phenol	Ferric chloride test	+	+	+
Steroids	Liebermann Burchard			
Reaction Test	-	-	-	
Glycosides	Kellerkilliani test	-	-	-
Carbohydrates	Fehling's test	+	+	+

(+) indicates positive result and (-) indicates negative result

PLATE 1: Antibacterial activity using well diffusion method

Hot aqueous extraction



Staphylococcus sp., *Escherichia coli*

Cold aqueous extraction



Staphylococcus sp., *Escherichia coli*

produced silver nanoparticles were ascertained by X-ray diffraction examination. The Shimadzu MODEL XRD-6000 equipment, which can be operated at 40 Kv of voltage with Cu K α X-radiation ($\lambda=0.15418$), was coated with about 200 mg of green produced silver nanoparticles. The size of the particles was measured using the Debye-Scherrer equation [$D = k\lambda/\beta\cos^*\theta$]. Akintelu, Bo and Folorunso, (2020).

Antibacterial Activity of Green Synthesized Silver Nanoparticles: Using Muller Hinton Agar media at 37°C for 24 hours, the antibacterial activity of combinatorial silver nanoparticles at a concentration of 500 mg/ml was assessed using the agar well diffusion method. The diameter of the zone of inhibition was used to measure the development of microorganisms. Nair, Kalariya and Chanda, (2005).

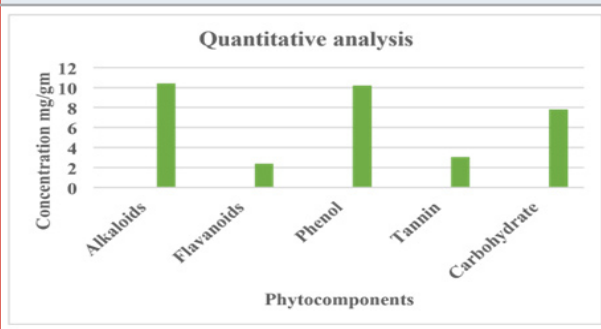
RESULTS AND DISCUSSION

Antibacterial Activity of Combinatorial Leaf Extract: The bactericidal activity of combination aqueous (hot and cold) extracts of *Adhatoda vasica*, *Aegle marmelos*, and *Cassia fistula* was evaluated at five distinct concentrations. The antibacterial activity was evaluated using the agar well diffusion method shown in plate no 1. The average zone of inhibition for each isolate was used to illustrate the results in both hot and cold aqueous extracts. The zone in Seemaisamy

Structure Elucidation of Green Synthesized Silver Nanoparticles: The crystalline size and structure of green

et al., (2019) was similar to the aqueous extract of *C. fistula*, *A. vasica* and *A. marmelos* of *Staphylococcus sp.*, and *Escherichia coli*.

Figure 1: Quantitative phytochemical analysis



Qualitative Phytochemical Analysis: *Aegle marmelos*, *Adhatoda vasica*, and *Cassia fistula* were the three different plants that underwent qualitative phytochemical study. The majority of the phytoconstituents in the leaves were alkaloids, flavonoids, protein, phenol, and carbs. *Cassia fistula* aqueous extract was subjected to a qualitative phytochemical examination. Alkaloids, flavonoids, protein, phenol, and carbs were found in a plant. Similar results were observed in Ali, (2014). Protein, carbohydrates, alkaloids, flavonoids, and saponins are all present in the *Adhatoda vasica* aqueous extract. Comparable outcomes were documented in Patel and Patel, (2023). *Aegle marmelos*'s aqueous extract contains protein, carbs, tannin, alkaloids, and flavonoids. Results for Ratnampally and Venkateshwar (2017) were comparable. Table 1 displays the results of the qualitative phytochemical investigation.

Table 2. Hemolytic activity of combinatorial leaf extract

DIFFERENT RATIOS OF AQUEOUS (HOT & COLD) EXTRACTS	RESULT
Hot (C.f:A.v:A.m-1:1:1)	No hemolysis
Hot (C.f:A.v:A.m- 0.5:0.5:1)	No hemolysis
Hot (C.f:A.v:A.m-1:0.5:0.5)	Hemolysis
Hot (C.f:A.v:A.m- 0.5:1:0.5)	Hemolysis
Hot (C.f:A.v:A.m- 0.5:0.5:0.5)	Hemolysis
Cold (C.f:A.v:A.m- 1:1:1)	Hemolysis
Cold (C.f:A.v:A.m- 0.5:0.5:1)	Hemolysis
Cold (C.f:A.v:A.m- 1:0.5:0.5)	Hemolysis
Cold (C.f:A.v:A.m- 0.5:1:0.5)	Hemolysis
Cold (C.f:A.v:A.m- 0.5:0.5:0.5)	Hemolysis

C.f – *Cassia fistula*; A.v- *Adhatoda vasica*; A.m- *Aegle marmelos*

Figure 2: UV Spectroscopy analysis of silver nanoparticles

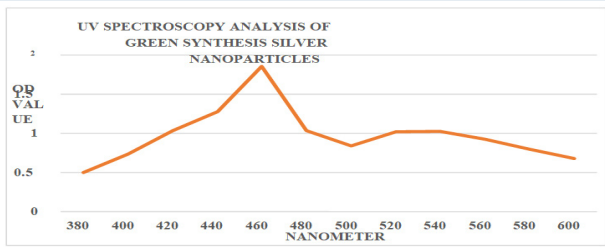


Figure 3: Ftir analysis of silver nanoparticles

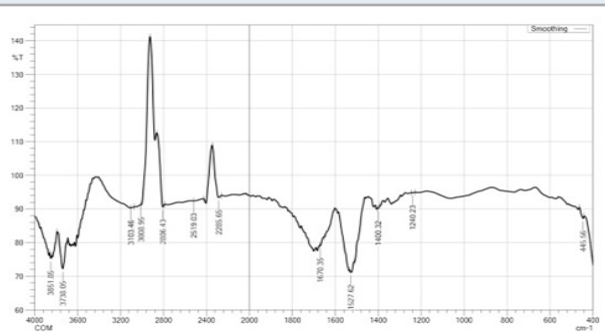
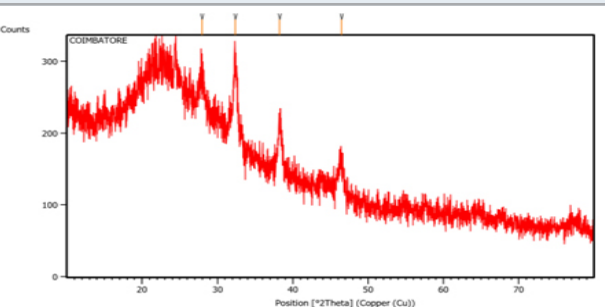


Table 3. Ftir analysis of silver nanoparticles

Frequency cm-1	Bond	Functional group
3851.85	O-H stretch, free hydroxyl	Alcohols, Phenols
3738.05	O-H stretch, free hydroxyl	Alcohols, Phenols
3103.46	=C-H stretch	Alkenes
3008.95	=C-H stretch	Alkenes
2806.43	H-C=O:C-H stretch	Aldehyde
2519.03	O-H stretch	Carboxylic acid
2285.65	O=C=O stretch	Carbon dioxide
1670.35	-C=C- stretch	Alkenes
1527.62	N-O asymmetric stretch	Nitro compounds
1400.32	C-C stretch	Aromatics
1240.23	C-N stretch	Aliphatic amines
445.56	C-Br stretch	Alkyl halides

Figure 4: Xrd analysis of silver nanoparticles



Quantitative Phytochemical Analysis of Combinatorial Leaf Extract: The quality of the phytochemical components contained in the leaf extracts from *Cassia fistula*, *Adhatoda vasica*, and *Aegle marmelos* was assessed. The phytochemical components were examined using the hot aqueous extract at a 1:1:1 concentration is tannins (3.036), phenol (10.2), alkaloids (10.4 mg/gm), flavonoids (2.4 mg/gm), and carbohydrates (7.8%). It was found that the flavonoid content was lower, and the alkaloid concentration was higher than other phytochemical components. Priya et al.,(2010) offered comparable explanations. The findings of a quantitative phytochemical analysis are displayed in Figure 1.

Hemolytic Activity of Combinatorial Leaf Extract: To assess their cytotoxic effect, combinatorial leaf extracts were examined for hemolytic activity in blood agar plates. The findings indicate that the hemolytic activity of plant extracts was demonstrated by the concentrations of hot

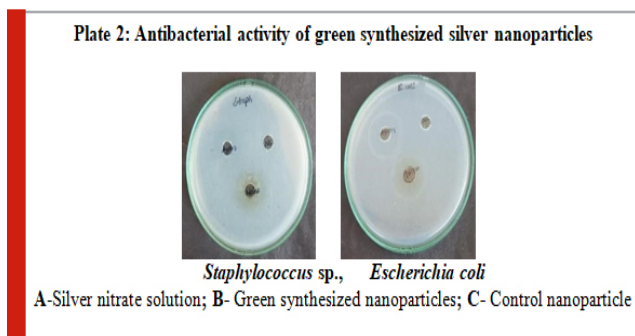
shift from yellow to brown signifies the formation of silver nanoparticles. With the use of UV-visible spectroscopy, it was further verified. The color shift from yellow to brown indicated the presence of silver nanoparticles. There was a correlation between the outcomes with Pang et al.,(2020).

Characterization of Silver Nanoparticles Using Combinatorial Leaf Extract Uv Absorption Spectrophotometric Analysis of Green Synthesis of Silver Nanoparticles: A UV absorption spectrophotometer was used to absorb the green produced silver nanoparticles. The peaks demonstrate that the creation of silver nanoparticles was indicated by the combinatorial leaf extract. The observed silver nanoparticles were in the 380–600 nm range. The synthesis of nanoparticles at 460 nm was indicated by the largest peak, which also showed the formation of nanoparticles at that wavelength. The descending peaks were anticipated. In Lakkim et al.(2020, the same findings were discussed. The outcomes of UV absorption spectroscopy are displayed in Figure 2.

Fourier Transform Infra Red Spectrophotometric Analysis of Green Synthesized Silver Nanoparticles: The functional group of the nanoparticles determines their stability and activity. In order to determine the various functional groups present in green produced silver nanoparticles, FTIR analysis was carried out. Peaks were used to represent the functional groups. The several functional groups, including alcohols, phenols, alkenes, carboxylic acids, nitro compounds, aromatics, aliphatic amines, aldehyde, carbon dioxide, and alkyl halides, are indicated by the green produced silver nanoparticles. The formation of silver nanoparticles was announced by the following functional groups. Although it serves as a capping and reducing agent throughout the silver nanoparticle process, the leaf extract was primarily covered with the nanoparticles. It was determined by the investigation that the leaf extract was used to create the silver nanoparticles. The combinatorial leaf extract was used to create silver nanoparticles, according to the results of the FTIR study. Kale and Dandge (2020) stated that green produced silver nanoparticles had the same functional groups. The FTIR analysis is displayed in Figure 3 and Table 3.

X Ray Diffraction of Combinatorial Leaf Extract: XRD analysis was used to examine the green produced silver nanoparticles amorphous and crystalline structures. The primary peaks and associated planes were identified by comparing the XRD data with the JCPDS file 040783. The peaks that were found were 38.269 for (111) and 46.442 for (200). In the (111) plane, the green produced silver nanoparticles' face-centered cubic (fcc) shape was clearly visible. According to Wilson and Venkateshwari (2022), the planes were a sign that silver nanoparticles were forming. Figure 4 illustrates the existence of XRD analysis structural elucidation.

The size of the green produced silver nanoparticles was determined using the Debye-Scherrer equation, $D = k\lambda / \beta \cos\theta$. Green produced silver nanoparticles ranged in size from 15 to 30 nm, with the greatest being 181.36 nm and



(0.5:1:0.5, 1:0.5:0.5, 0.5:0.5:1) and cold (1:1:1, 1:0.5: 0.5, 0.5:0.5:1, 0.5:1:0.5, and 0.5:0.5:0.5). wherein the plant extracts' non-hemolytic activity was demonstrated by the hot (1:1:1 and 0.5:0.5:1). Plant extracts with non-hemolytic activity were used for other purposes Kalita et al. (2011), and the hemolytic activity of a plant extract in water was examined. Mathur et al., (2011) talked about the plant's aqueous extract's non-hemolytic action, Table 2 presents the results.

Table 4. Antibacterial activity of silver nanoparticles

Organisms	Zone Of Inhibition(Mm) Silver Nanoparticle		
	AgNPs	AgNO3	NC
<i>Staphylococcus sp.</i> ,	13 mm	R	R
<i>Escherichia coli</i>	15 mm	10 mm	R

R- Resistant; AgNPs- Silver nanoparticles; AgNO3- Silver nitrate solution; NC- Negative Control

Green Synthesis of Silver Nanoparticles Using Combinatorial Leaf Extract: Silver nanoparticles were created using the green synthesis technique. For the synthesis of silver nanoparticles, a nonhemolytic ratio of hot 0.5:0.5:1 was used from the earlier investigation. The color

the smallest being 159.76 nm. According to these findings, the current investigation showed rapid estimates, with the smallest size created at 15.71 nm, which corresponds to (111), and the highest size of synthesized silver nanoparticles from the combinatorial leaf extract being 23.63 nm (200).

Antibacterial Activity of Green Synthesized Silver Nanoparticles: Combinatorial leaf extract's silver nanoparticles antibacterial properties were investigated. Green produced silver nanoparticles demonstrated a 13 mm zone of inhibition for *Staphylococcus* sp. and a maximal zone of 15 mm for *Escherichia coli*. The findings make it abundantly evident that silver nanoparticles have strong antibacterial action through zone formation. Zones obtained by Khadka et al. (2020) were nearly identical. Table 4 and Plate 2 displayed the findings.

CONCLUSION

The hot and cold leaf extracts show the existence of different phytochemical components, according to the research mentioned above. In a hot 1:1:1 aqueous extract, the combinatorial leaf extract of all three plants included 10.4 mg of alkaloids, 10.2 mg of phenol, 3.03 mg of tannin, 2.4 mg of flavonoids, and 7.8 mg of carbohydrates. Combinatorial leaf extracts are used to create green-manufactured silver nanoparticles, which display a wide variety of phytochemical components. When compared to plant-based antibacterial agents, silver nanoparticles showed increased antibacterial activity. When compared to other commercial antimicrobial medications, the green synthesis approach has several advantages, including being easy to use, quick, inexpensive, and most significantly, environmentally benign. Green-manufactured silver nanoparticles successfully inhibit the microorganisms.

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