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Comparative Study of Mercuric Oxide Nanoparticles Synthesized using Ferulic Acid and Curcumin: Characterisation and Antibacterial Activity

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Abstract

This study reports the green synthesis of mercuric oxide nanoparticles (HgO-NP's) using two natural phytochemicals Ferulic acid and curcumin as reducing and stabilizing agents. The formation of HgO NPs was initially evidenced by colour transitions from pale yellow to brownish red with ferulic acid and bright yellow to reddish brown with curcumin. UV-Visible spectroscopy confirmed the formation nanoparticles with ferulic acid mediated HgO-NPs exhibiting a sharp absorption peak at 320nm, while curcumin mediated synthesis resulted in a broader peak between 275-450 nm. FTIR analysis identified functional groups responsible for the formation and stabilization of the nanoparticles with prominent peaks for –OH (3450 -3510 cm^{-1}) , C=O stretching (1682 cm^{-1} for ferulic acid and 1625 cm^{-1} for curcumin) and HgO vibrations (540-550 cm^{-1}).Transmission Electron Microscopy (TEM) analysis revealed that ferulic acid mediated HgO-NP's were larger (5-50 nm)and more aggregated whereas curcumin mediated HgO-NP's were smaller (2-20 nm) with better dispersion and more defined spherical morphology. Antibacterial studies showed enhanced activity of both types of HgO-NP's against *E. coli*, *S. aureus*, *B. cereus* and *P.aeruginosa* compared to conventional antibiotic, with curcumin mediated HgO-NP's exhibiting superior activity against *P.aeruginosa*. These findings highlights how phytochemical structure diversity can be utilised to tune nanoparticles properties and their potential applications in diverse fields such as biomedical, environmental and agriculutural domains.

Introduction

Nanotechnology has revolutionised materials science offering innovative solutions across diverse fields such as medicine, catalysis and environmental remediation[1,2]. Metallic nanoparticles with their unique properties, arising from their nanometer dimensions, find wide application in these areas. [3,4]. However, conventional fabrication methods often require harsh conditions and toxic chemicals raising environmental concerns. Green chemistry approaches for nanoparticle synthesis is crucial, as they focus on minimization or total elimination of waste production promoting sustainable processes [5]. These efforts aim at utilization of nontoxic chemicals, environmentally benign solvents, and renewable materials, are some of the key issues that merit important consideration in a green synthetic strategy[6]. Among the green synthesis methods, fabrication of metallic nanoparticles using various plants system is an eco-friendly approach[7]. Plant derived phenolic acids such as Caffeic, ferulic, p-coumaric and sinapic acids are attractive reducing and capping

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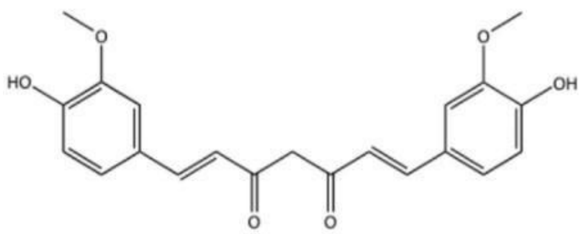
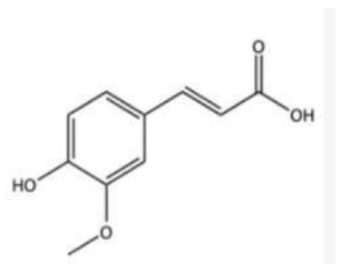
agents for nanoparticle synthesis (Bravo 1998, Balasundram et al. 2006) due to their anti-inflammatory, anti-bacterial and anti-cancer properties[8]. Phenolic acids are significant components of fruit and vegetables, which play an important role in colour stability, aroma profile and antioxidant activity[9]. Another widely studied phytochemical called curcumin; which is a polyphenol also exhibits strong antimicrobial and anticancer activity. In parallel, the increasing threat of antibiotic resistance and environmental pollution by heavy metal ions like mercury necessitates the development of novel materials with enhanced antibacterial activity and catalytic efficiency[10]. While metal NP's are known for their unique physicochemical characteristics, metal oxide NP's are valued for their wider range of applications ranging from electronics to medicine[11]. Mercuric oxide is a widely used compound, particularly in batteries and chemical synthesis processes. Despite its usefulness, the toxicity of mercury even at lower levels, raises significant health and environmental concern[12,13]. Therefore, there is a need to develop efficient and environmentally friendly method to remove mercury from the contaminated sources and its environmental footprint. Metal oxide nanoparticles, particularly mercuric oxide have shown significant antibacterial and catalytic activity due to their unique properties[10,14]. In this study two phytochemicals representing distinct class of natural phenolics curcumin, a polyphenol from curcumin longa and ferulic acid, a widely occuring phenolic acid were selected for the green synthesis of mercuric oxide nanoparticles (HgO-NP's).The synthesized nanoparticles will be characterised using UV-Visible Spectroscopy, FTIR spectroscopy and TEM, and their antibacterial activity also will be sytematically evaluated.



Curcumin



Ferulic acid

Figure 1. Structures of Curcumin and Ferulic acid

2.1 Experimental

2.1.1 Materials

All chemicals and reagents were used of analytical grade. Mercuric acetate, curcumin and ferulic acid were sourced from Sigma Aldrich.

Synthesis of HgO-NP's

Synthesis with Ferulic acid: To synthesize HgO-NP's, 10 ml aliquot of 5 mM mercuric acetate solution (10 ml) was transferred into a 250 ml conical flask. Subsequently, 5ml of 5 mM ferulic acid solution was added dropwise to the mercuric acetate solution under gentle stirring. The reaction mixture was then heated at 60^{o}C with a constant stirring for 1 hour.

Following the reaction, the resulting HgO-NP's were separated by centrifugation at 5000 rpm for 15 minutes. The precipitate was washed three times with ethyl alcohol to remove impurities. The purified nanoparticles were then vacuum dried at room temperature, for subsequent characterisation.

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Synthesis with curcumin: HgO-NP's were synthesized using curcumin as the reducing agent following an identical procedure and concentration as described above, with curcumin solution substituted for ferulic acid solution.

Characterization of HgO-NP's

UV-Visible Spectroscopy: The HgO-NP's obtained were analysed using UV-Visible Spectroscopy**.** The UV-Visible spectrum of the HgO-NP's was obtained using Shimadzu UV 3600 spectrophotometer over the wavelength range of 200-800 nm, with a resolution of 1 nm, for providing insights into electronic transitions of NP's.

FT-IR Spectroscopy: The organic moieties of the phytochemicals responsible for the reduction and stabilisation of HgO-NP's were examined using Thermo Nicolet Avtar 370 FTIR instrument over the wavelength range of 400-4000 cm^{-1}.

TEM Analysis: Morphology of HgO-NP's was studied using Jeol Jem 2100 Transmission Electron Microscope with an operating voltage of 200 kV.

Antibacterial Assay of HgO-NP's by Well Diffusion Method

The antimicrobial activity of HgO-NP's was evaluated against four bacterial strains: two of them are gram positive: *Bacillus cereus*, *Staphylococcus aureus*, and two are gram negative: *Escherichia choli* and *Klebsiella pneumoniae*. Despite their difference all four strains are clinically relevant multidrug resistant pathogens. All the strains were obtained from the Cashew Export Promotion Council of India (CEPCI), Kollam, Kerala. Mueller Hinton broth (1.4 g, HiMedia Laboratories) was dissolved in 50 ml of distilled water, inoculated with bacterial strains and incubated at 37^0C for 24 hours. For the antibacterial assay, 1.2 g nutrient agar in 50 ml distilled water, poured into four pre-sterilized Petri plates (55 x 15 mm), and allowed to solidify. The bacterial strains were spread using a sterilized cotton swab. After 5 minutes, 4 wells having 8 mm diameter were punched in each plate, with a sterile cork-borer. 50 µL each of HgO-NP's dispersed in water (5 mM), negative control (sterilized water), positive control (Ampicillin -10 µg/mL) and Ferulic acid (5 mM) were introduced into the wells. Plates were incubated for 24 hours at 37^0C. After the incubation, the diameter of inhibition zones was measured.

3. Results and Discussions

The formation of mercuric oxide nanoparticles was identified by the colour change that occurred when HgO-NP's were formed using ferulic acid and curcumin. The colour transition observed with ferulic acid shifted from pale yellow to brownish red, whereas curcumin mediated synthesis resulted in a change from bright yellow to reddish brown, indicating differences in their reducing capabilities and stabilisation. The presence of mercuric oxide nanoparticles was confirmed by UV-Visible spectroscopy. FTIR spectroscopy provided information about different functional groups that are responsible for the formation and stabilisation of mercuric oxide nanoparticles. Morphology of the mercuric oxide nanoparticles were identified using TEM analysis. Antibacterial activity of the mercuric oxide nanoparticles were studied using well-diffusion method respectively.

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3.1 UV-VISIBLE SPECTROSCOPY

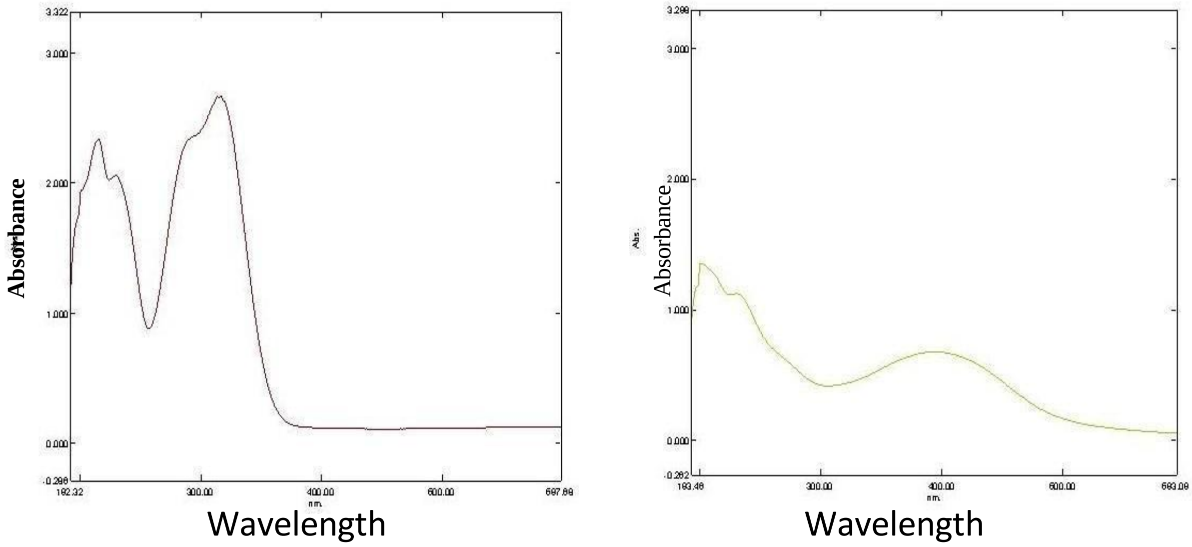

Figure 2 : UV -Visible Spectrum Of Mercuric Oxide Nanoparticles Using a) Ferulic acid b) Curcumin

Figure 2 a and 2b depicts the UV-Visible spectra of the mercuric oxide nanoparticles using ferulic acid and curcumin respectively. The HgO-NP's synthesized with ferulic acid exhibited sharp and intense absorption peak at 320 nm suggesting a smaller particle size. In contrast, a broader peak obtained with curcumin in the range of 275 to 450 nm, may be due to variations in nanoparticle size distribution. However the decrease in absorbance after 350 nm indicates the characteristic behaviour of HgO-NP's reduced by ferulic acid aligning with the previously reported values.[15]

3.2 FTIR SPECTROSCOPY

The FTIR spectra of HgO-NP's synthesized using ferulic acid and curcumin depicted in Figure 3 and Figure 4 respectively, showed distinct differences in their functional group interactions. Broad peaks were obtained for both samples in the range of 3450 -3510 cm^{-1} corresponding to –OH functional groups[16].

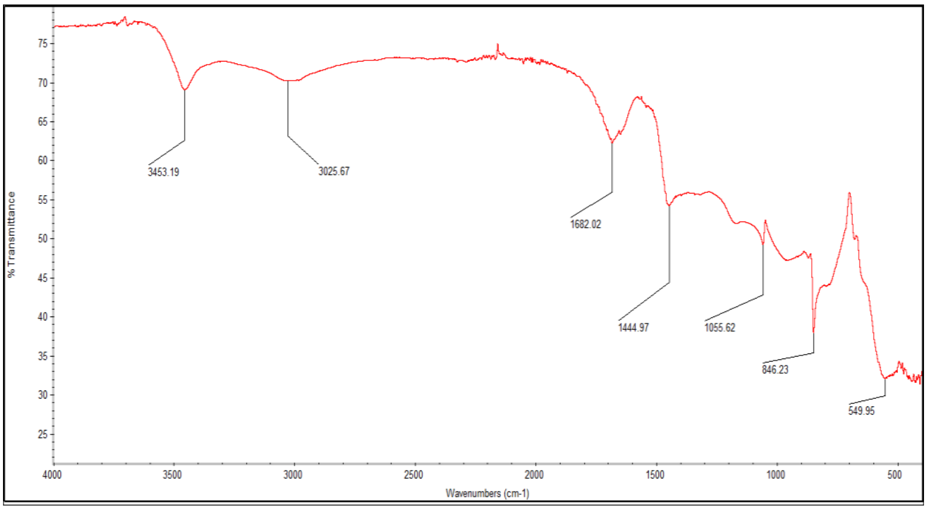

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Figure 3. FTIR Spectrum of mercuric oxide nanoparticles synthesized using ferulic acid

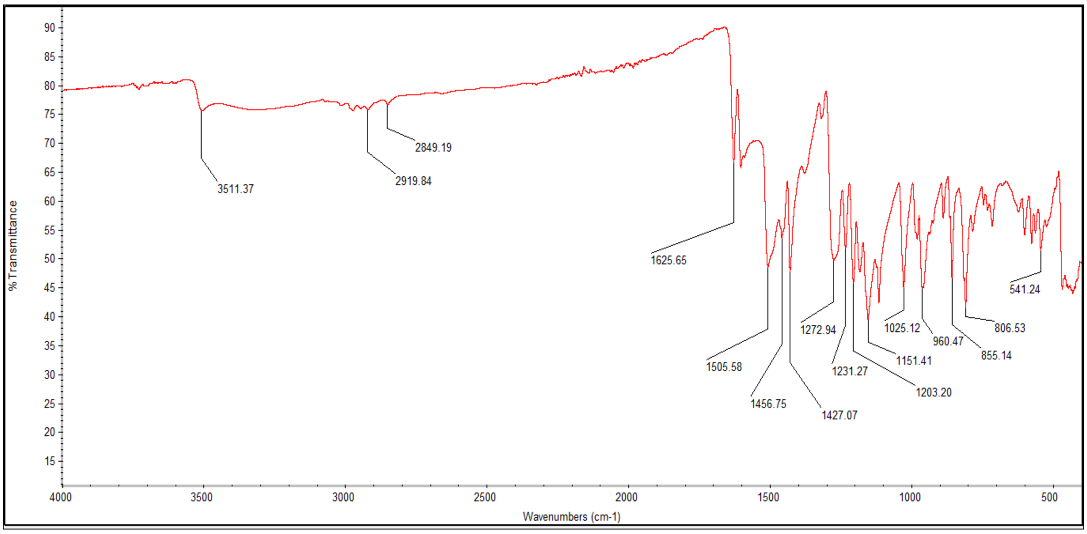

Figure 4. FTIR Spectrum of mercuric oxide nanoparticles synthesized using curcumin

The characteristic CH stretching vibrations were observed in both cases appearing at 3025 cm^{-1} and around 2919 cm^{-1} and 2849 cm^{-1} for curcumin. A strong C=O stretching peak was observed at 1682 cm^{-1} for ferulic acid and 1625 cm^{-1} for curcumin suggests the interaction of carbonyl group in the nanoparticle formation[17]. The aromatic C=C stretching vibrations were observed near 1444 cm^{-1} for ferulic acid and at 1505, 1456 and 1427 cm^{-1} for curcumin, point towards the conjugation of organic moieties with the nanoparticles[18]. Curcumin mediated NP's displayed significant stretching bands in the range of 1200-1300 cm^{-1}, indicates the involvement of ether linkage[19]. The strong peaks in the region of 540-550 cm-1 in both samples could be due to the Hg-O stretching vibrations, supporting the successful synthesis of HgO-NP's[20].

3.3 TEM ANALYSIS

Figure 5 presents the TEM images of HgO-NP's derived using ferulic acid (a-c) and curcumin (d-f). The NP's synthesized with ferulic acid (a-c) appears to be larger and aggregated with an average particle size ranging from 5 to 50 nm[21].

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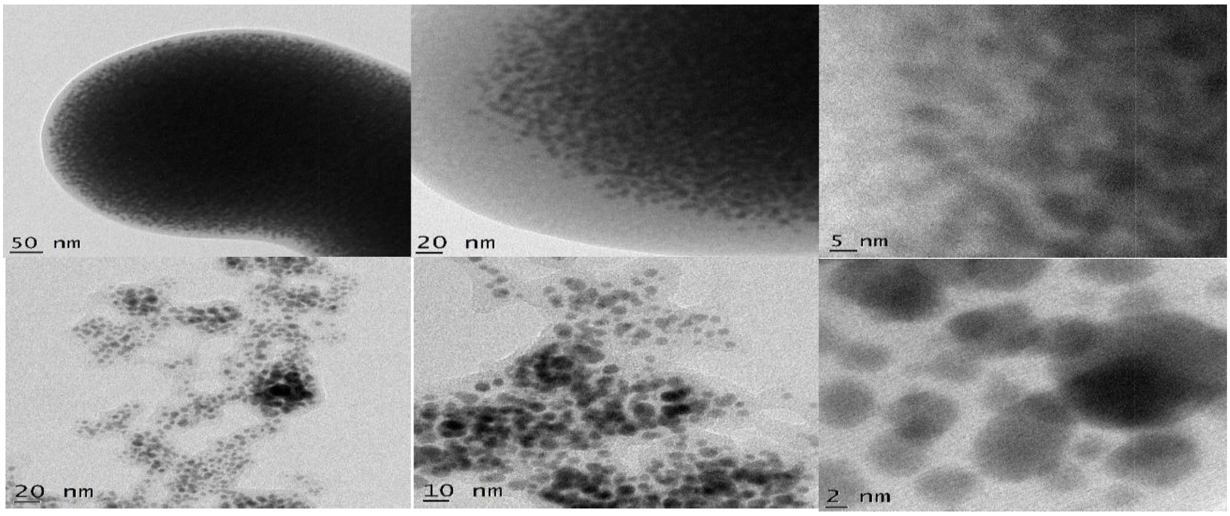

Figure 5 : TEM Images Of Mercuric Oxide Nano Particles Using (a-c) Ferulic Acid And (d-e) **Curcumin**

In contrast, the NP's synthesized with curcumin (d-f) are more uniformly dispersed and exhibit a smaller size with an average size between 2 and 20 nm. From the high resolution images it can be inferred that curcumin derived NP's are less aggregated with more defined spherical morphology[22]. The observed differences in particle size and dispersion suggest that the nature of reducing and stabilizing agent significantly influences the structure and morphology of the HgO-NP's.[23]

3.4 Antibacterial Assay Of Mercuric Oxide Nanoparticles

Figure 6a presents the antibacterial activity images of ferulic acid and curcumin mediated HgO nanoparticles induced zone of inhibition in agar plates. Mercuric oxide nanoparticles synthesised using both ferulic acid and curcumin exhibited strong antibacterial properties compared to the standard antibiotic ampicillin (positive control). From Table 1, it can be seen that among the tested strains B.cereus showed the least susceptibility, while P. **auruginosa** was the most susceptible to the nanoparticles . The histogram given in Figure 6 b. shows that the pure phytochemicals exhibited moderate antibacterial activity, however nanoparticle along with the capping agent showed enhanced activity. Ferulic/ HgO is observed to be broadly effective against E.coli and S.aureus. However curcumin/ HgO showed superior activity against P.auruginosa (2.9 cm) likely due to its smaller size and stronger curcumin metal interactions. The absence of inhibition zones in the negative control confirms the antibacterial effect of the nanomaterial[24].

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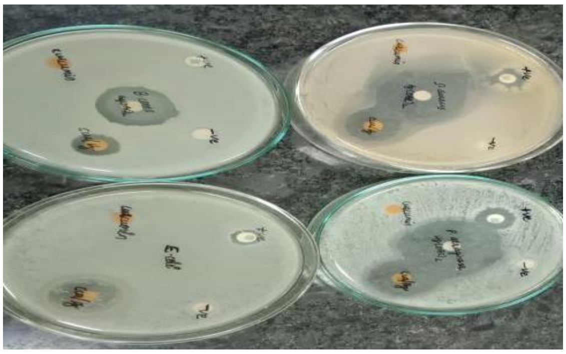

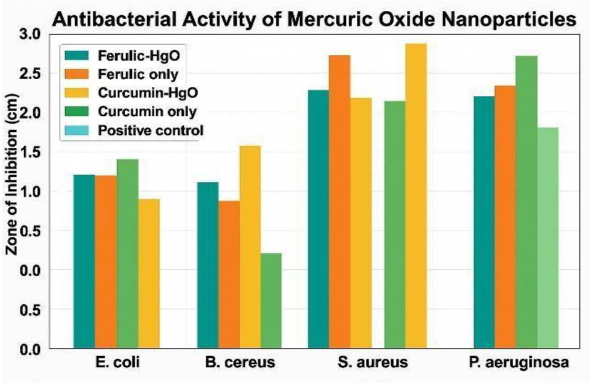

Figure 6 a: Antibacterial activity of ferulic acid and curcumin mediated HgO nanoparticles against bacterial strains E.coli, B.ceres, S.aures, and P.aeruginosa through Zone of inhibition in agar plates b) Comparative analysis of antibacterial analysis of ferulic acid and curcumin mediated HgO nanoparticles against bacterial strains E.coli, B.ceres, S.aures, and P.aeruginosa through Zone of inhibition

Table 1: Antibacterial activity (Zone of inhibition in cm) of ferulic acid and curcumin mediated HgO nanoparticles

Sl. No	Strain	Ferulic acid/HgO	Ferulic acid only	Curcumin/ HgO	Curcumin only	Positive control	Negative control
1	E.coli	2.1	2.2	2.2	1.1	0.5 (FA)/ 0 (Cur)	0
2	B.ceres	1.2	1.1	1.1	0.1	0.4 (FA)/ 0 (Cur)	0
3	S.aures	2.3	2.4	2.4	0.8	0.8 (FA)/ 0 (Cur)	0
4	P.aeruginosa	2.1	2.2	2.2	1.6	0.3 (FA)/ 0 (Cur)	0

Conclusions

In this study, we successfully synthesized HgO-NP's using green synthesis approach with ferulic acid and curcumin as reducing and stabilizing agents. The formation of HgO-NP's was confirmed through multiple characterisation techniques like UV-Visible spectroscopy, FTIR and TEM which revealed distinct properties depending on the nature of the phytochemical used. UV-Visible spectroscopy confirmed the successful formation of HgO-NP's with characteristics absorption pattern, at 320 nm with ferulic acid versus a broader peak between for curcumin mediated synthesis. FTIR studies showed significant peaks indicating the involvement of key functional groups such as hydroxyl, carbonyl and aromatic moieties of both phytochemicals in the formation of nanoparticles. TEM images confirmed the formation of HgO-NP's with distinct properties where curcumin mediated nanoparticles were smaller (2-20 nm) in size and had better dispersion when compared to ferulic acid mediated nanoparticles (5-50 nm). Both type of nanoparticles exhibited strong antibacterial activity against *E. coli*, *S. aureus*, *B. cereus* and *P.aeruginosa* with curcumin mediated HgO-NP's showing particularly strong efficacy against

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P.aeruginosa. These findings highlight the potential of these green synthesized HgO-NP's as antimicrobial agents for various applications in healthcare, environmental remediation and material science.

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