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Green Synthesis of Copper Oxide Nanoparticles Mediated by Aqueous Leaf Extracts of *Leucas aspera* and *Morinda tinctoria*

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Abstract: Copper oxide nanoparticles were successfully synthesized using the aqueous leaf extracts of *Leucas aspera* and *Morinda tinctoria* plant material with copper sulfate ($CuSO_4.5H_2O$) as the precursor. The crystalline nature and morphology of the synthesized sample were identified using XRD and SEM analytical instrumentation and found that the crystal was in the monoclinic phase, and the average particle size was estimated as 30.32nm and 18.72nm for both the samples. The functional groups were identified using FTIR spectroscopy, and the strong absorption peak at 620 cm^{-1} and 615cm^{-1} confirms the presence of Cu-O vibration. The optical bandgap of the plant leaf extract mediated CuO particles was calculated based on the results obtained from UV-Vis spectroscopy and found that the value of the energy gap is 5.6eV and 3.16eV. The antibacterial activity of plant samples was carried out by the disc diffusion method. The test compounds' concentrations were taken in DMSO and used in the concentration of 500 µg and 1000 µg /disc. The zone of inhibition formed by the prepared CuO nanoparticles was good and compared with Amikacin's standard value. The study reports the plant leaf extract mediated CuO nanoparticles might find suitable application in the field of nanotechnology.

Keywords: *Leucas aspera*; *Morinda tinctoria*; green synthesis; copper oxide; antibacterial.

1. Introduction

Among metal oxides, copper oxide has attained more attraction in the last few decades due to its characteristic properties such as high-temperature superconductivity, electron correlation effects, spin dynamics, etc. CuO NPs have been used in various fields, including industrial engineering, agriculture, and technical fields [1]. Recently the field of agriculture focused on the impact of certain minor elements on the economy of plants. Bionanotechnology utilizes biological principles and chemical approaches to yield nano-sized particles with specific functions. The morphology and size effect contribute a maximum for developing new tools and ideas for the technical approach in various fields [2]. For instance, the fabrication of metal oxide nanoparticles like silver and gold finds some difficulties and is cost-effective, whereas synthesizing CuO NPs is eco-friendly and finds useful properties and is achievable at lower costs than silver and gold. Moreover, the specific properties like catalytic, optical, electrical, antibacterial, and antifungal applications exhibited by CuO NPs are excellent compared to other metals. Biosynthesis of CuO NPs by plant leaf extracts such as *Euphorbia nivulia* and *Nerium oleander* has been reported [3], and plants' potential as biological materials for the synthesis of CuO NPs is yet to be focused and explored thoroughly. *Leucas aspera* is commonly known as Thumbai (in Tamil) and is distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally as an antipyretic and insecticide [4]. The leaves are useful in chronic rheumatism, psoriasis, and other chronic skin eruptions. The steam is used for inhalation in conditions like nasal congestion, cough, cold, fever, headaches, etc. The plant is reported to contain various phytochemicals such as oleanolic acid, ursolic acid, α-sitosterol, β-sitosterol, reducing sugars, diterpenes, glycosides, (-)-isololiolide, linifolioside, nicotine, macelignan, acacetin, apigenin, myristargenol B, machilin C, and (-)-chicanine. In addition to the above-said compounds, steroids, triterpenes, phenols, flavonoids, tannins, and long-chain aliphatic compounds were also reported [5]. *Morinda tinctoria* is another plant material used in this study; it is locally known as "*Togaru*" and commonly known as Indian mulberry or *aal* or *nunaa* in India. The plant is well known for its therapeutic use in traditional systems of medicine like Ayurveda and Siddha. The leaves are used internally as a tonic and febrifuge. There are reports which showed antibacterial and antifungal activities [6]. The ashes of *Morinda tinctoria* leaves were also reported to act as bioabsorbents in controlling ammonia pollution in wastewaters [7].

In this study, we report the green synthesis of Copper oxide (CuO) NPs mediated by aqueous leaf extracts of *Leucas aspera* and *Morinda tinctoria* plant. The study's objective is to compare the effects of the bio reducing compounds present in the leaf extracts in forming the CuO nanoparticles. The same procedure was adopted for both the plant leaf extracts mediated synthesis of CuO nanoparticles with copper sulfate as the precursor material. The synthesized CuO NPs were characterized using various analytical techniques like XRD, FTIR, SEM, UV-Vis, and antibacterial studies.

2. Materials and Methods

2.1. Preparation of leaf extracts

Experimental leaves of *Leucas aspera* and *Morinda tinctoria* were collected from the rural area around Tirupattur district, Tamil Nadu, India. The extract was prepared separately for each experimental leaf with the same procedure [8-9]. The extract was prepared by using fresh *Leucas aspera* leaves. The leaves were washed thoroughly in running water for few minutes and then washed with deionized water. About 25g of finely cut *Leucas aspera* leaves were taken and poured in to100 ml of deionized water and heated until the water attains 80 ºC for 20 min. The solution appeared as a light pale green color, and the extract was filtered through a sterile serene cloth and refiltered through Whatman No.1 filter paper to get a clear solution. The filtrate was collected and stored at 4 ºC for further use. The same procedure was adopted for the preparation of *Morinda tinctoria* leaf extract preparation.

2.2. Synthesis of copper oxide nanoparticles.

All the chemical reagents are of analytical grade and used without further purification. Nanoparticles of CuO were prepared using the *Leucas aspera* and *Morinda tinctoria* leaf extracts as a bio-reducing agent. $CuSO_4.5H_2O$ was used as the precursor, and about 0.37g was dissolved in 75 ml of deionized water and stirred well in the magnetic stirrer [10-12]. About 25 ml of fresh leaf extract of *Leucas aspera* was added into the prepared copper sulfate solution. The solution color changed from blue to pale yellowish-green, and on vigorous stirring, the solution changed its color to dark brownish-green [13-17]. The final solution was centrifuged well for 20 min at 10,000 rpm, and the solution is multi-washed by double distilled water and was poured into the Petri dish and kept in the air oven at 100 °C [18] for 2 hours for further study. A similar synthesis procedure was adopted for the *Morinda tinctoria* mediated CuO nanoparticles.

3. Results and Discussion

3.1. Powder X-ray diffraction analysis

The specimen was analyzed using the XRD technique to investigate the structure and nature of the synthesized CuO nanoparticles mediated by *Leucas aspera* and *Morinda tinctoria leaf extracts*. The recorded XRD pattern of the CuO is shown in Figure 1(a-b). The 2θ value ranging from 20º to 90º with intense peaks specific for Copper Oxide nanoparticles at 25.9°, 44.34°, 64.5°, and 77.7° were observed [19]. These peaks correspond to (200), (111), (200),(202), (220), and (311) planes, indicating that the particles are crystalline. The sharp bands of Bragg's peak authenticate that the particles are in the nano regime [20] and are stabilized by the bio reducing agents present in the leaf extracts of *Leucas aspera* and *Morinda tinctoria*. The planes are in good agreement with standard diffraction data (JCPDS-80-1916) and confirming the formation of a monoclinic crystalline structure [21]. The average particle size was estimated using Debye-Scherer Equation. The average particle size was found to be 30.32nm and 18.72 nm, respectively.

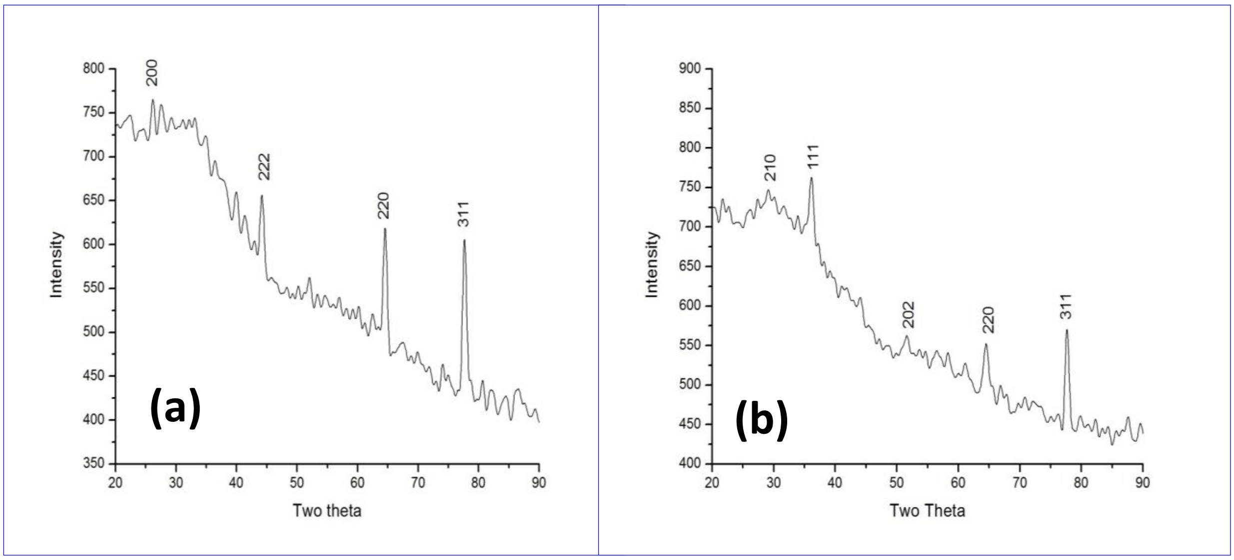

Figure 1. XRD Pattern of (**a**) *Leucas aspera* mediated CuO; (**b**) *Morinda tinctoria* mediated CuO.

3.2. FTIR analysis.

FTIR analysis was carried out to identify the functional groups, and the recorded spectrum is shown in Figure 2(a-b). About 16 absorption peaks were observed for both leaf extracts mediated CuO samples. It shows the interaction sites of the bio-reducing compounds of the leaf in the formation of bonding. The peaks at 3956 cm^{-1}, 3908 cm^{-1}, and 3758.65 cm^{-1} are due to OH bending and stretching of water molecules [22]; the peak at 3402.31 cm^{-1} is due to OH stretching of hydrogen-bonded alcohols and phenol compounds [23]. The peaks around 1627 cm^{-1} and 1630 cm^{-1} are due to unsaturated nitrogen, tannins, and alkaloids present in the leaf extracts [24]. The absorption peaks 2926.35 cm^{-1} and 2925 cm^{-1} were assigned to $-CH_2$ stretching mode in alkanes, and the peak at 2340.78 cm^{-1} assigned to –N=C=O stretching. The peak at 136 cm^{-1} and 1100 cm^{-1} shows C-N stretching of the aromatic amino group. A strong absorption peak at 1069 cm^{-1} is due to the aliphatic fluoro compounds [25]. The peak at 620.58 cm^{-1} and 615.45 cm^{-1} in both the samples confirms the formation of the metal oxide bond Cu-O.

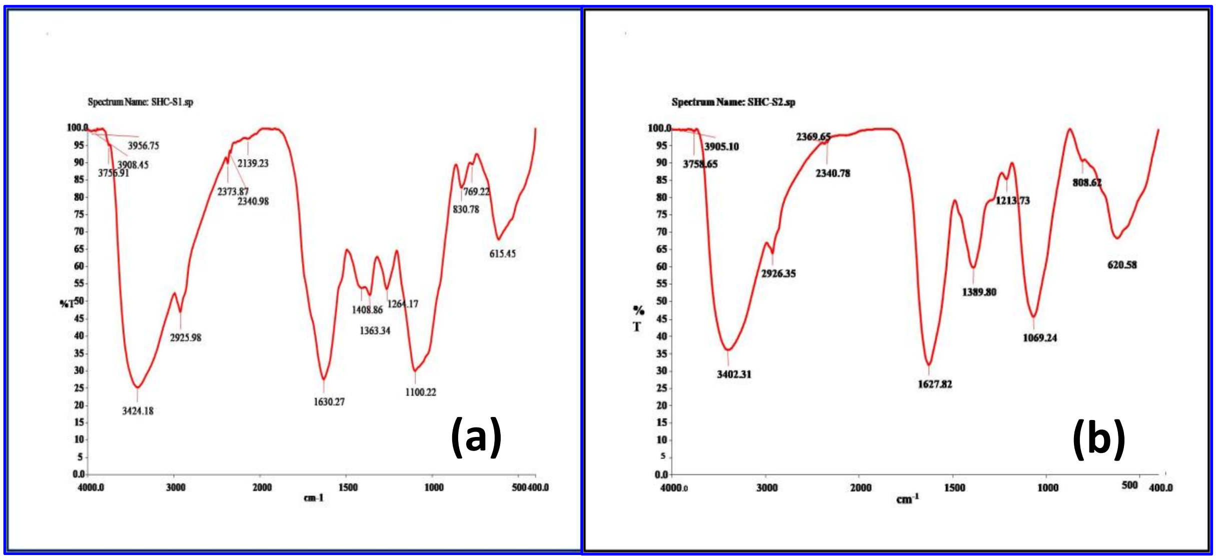

Figure 2. FTIR Spectrum of (**a**) *Leucas aspera* mediated CuO; (**b**) *Morinda tinctoria* mediated CuO.

3.3. UV-visible spectroscopic analysis.

The result obtained from UV-Visible spectroscopy analysis of both the leaf extract mediated CuO samples is given in Figure 3(a-b). The CuO NPs formation was confirmed from the peak at 319 nm in *Leucas aspera* leaf extract and 412 nm for the *Morinda tinctoria* leaf extract sample [26].

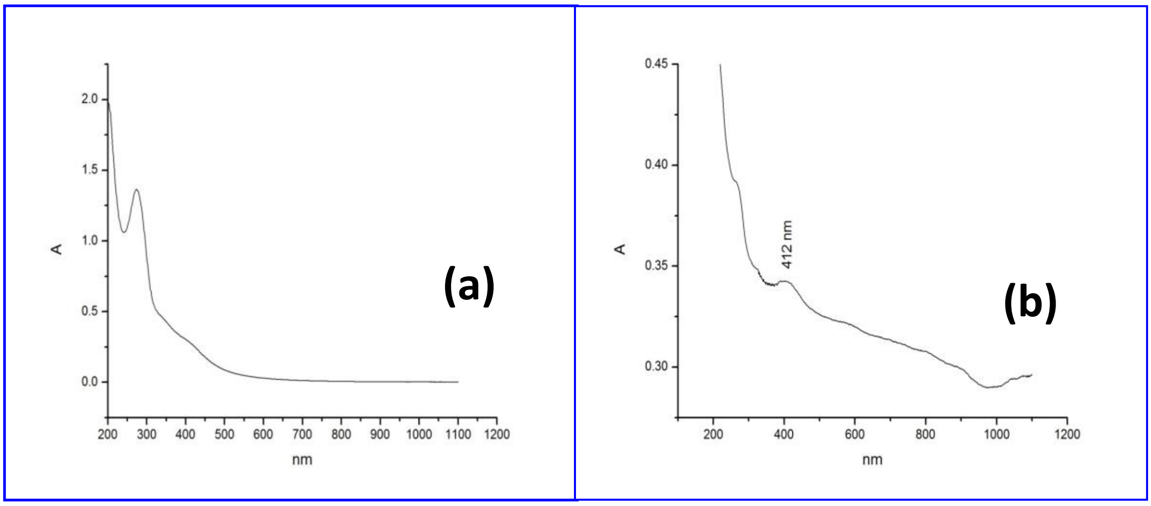

Figure 3. UV-Vis Spectrum of (**a**) *Leucas aspera* mediated CuO; (**b**) *Morinda tinctoria* mediated CuO.

These results agree with the reported investigations on plant leaf extract mediated CuO nanoparticles. It is attributed to the reason that the bio-reducing compounds present in the various plants modify the range of absorption and the optical activity in the UV-Vis region. Copper SPR effects decrease with time because of the oxidation of the synthesized copper [27].

The peak value was gradually decreased with the increase in particle size in the *Leucas aspera* leaf extract sample. The bandgap for the copper oxide nanoparticles is found to be 5.6 eV and 3.16 eV.

3.4. Morphological analysis

The morphological nature of the *Leucas aspera* and *Morinda tinctoria* leaf extract mediated CuO nanoparticles were analyzed using SEM analytical technique and is shown in Figure 4(a-b). The obtained results show that the particles were agglomerated, overlapped, and distributed non-homogeneously [28-32]. It may be due to the interaction of the bioactive and phytochemical compounds present in the leaf extracts. The SEM image shows some small spherical shape in the *Leucas aspera* leaf extract mediated sample and small cubical shapes in the case of the *Morinda tinctoria* mediated sample [33]. The variations in the sample's morphology prepared by the sampling procedure and exhibiting the different morphology and shape indicate that the formation of nanoparticles is dependent [34] on nature and the chemical compound present in the reducing/ capping agent of the plant materials taken for the investigation.

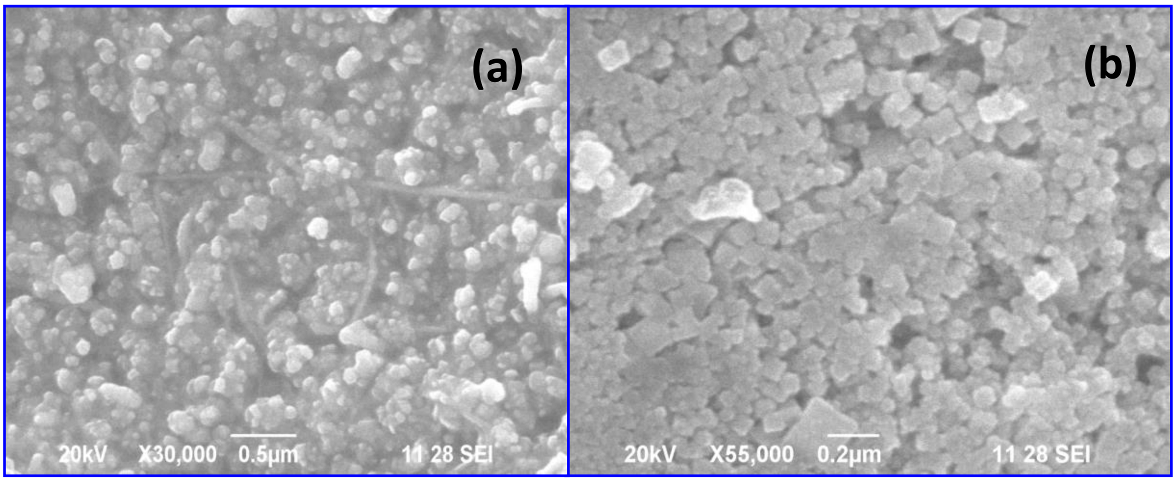

Figure 4. SEM image of (**a**) *Leucas aspera* mediated CuO; (**b**) *Morinda tinctoria* mediated CuO.

3.5. Antibacterial activity of prepared CuO nanoparticles.

The antibacterial activity of plant samples was carried out by the disc diffusion method and is shown in Figure 5(a-b). The test compounds' concentrations were taken in DMSO and used in the concentration of 500 µg and 1000 µg g/disc. The target microorganisms were cultured in Mueller–Hinton broth (MHB). After 24 h, the suspensions were adjusted to standard sub-culture dilution. The Petri dishes containing Muller Hinton Agar (MHA) medium were cultured with diluted bacterial strain. A disc made of Whatman No.1, diameter 6 mm, was pre-sterilized and was maintained in an aseptic chamber. Each concentration was injected into the sterile disc papers. Then the prepared discs were placed on the culture medium. Standard drug amikacin (30 µg) was used as a positive reference [35] standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 ºC for 24 h. The clear zone diameter around the disc was measured and expressed in millimeters as its anti-microbial activity. Both samples were screened for *in vitro* antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Bacillus subtilis* by disc diffusion technique compared with standard drug Amikacin (30 µg) [36]. The details of the organism used for the study and the inhibition zone for the *Leucas aspera* and *Morinda tinctoria* sample were calculated in mm and given in Tables (1-3).

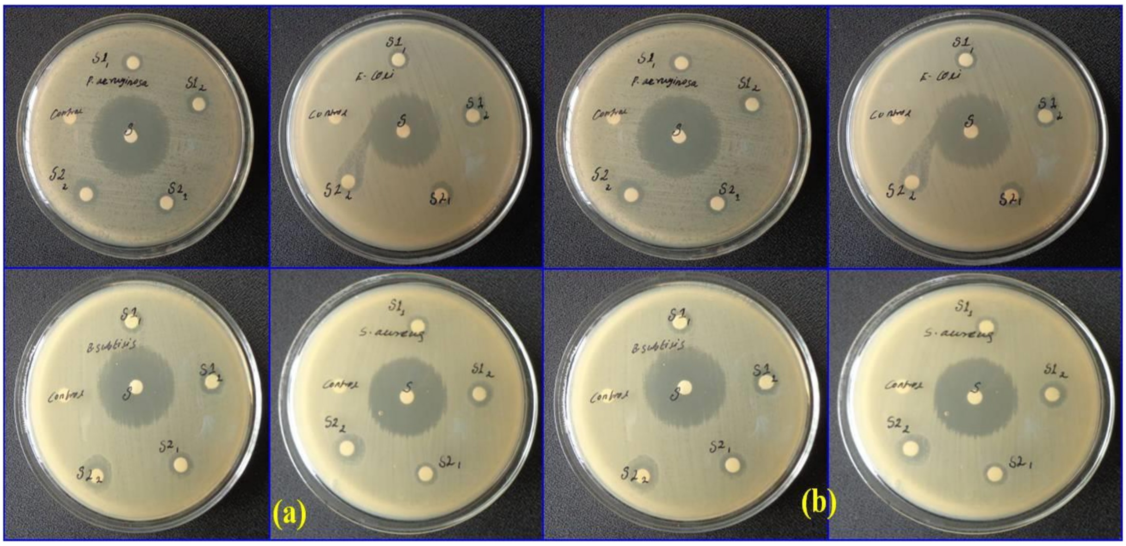

Figure 5. Antibacterial activity of (**a**) *Leucas aspera* mediated CuO; (**b**) *Morinda tinctoria* mediated CuO.

Table 1. Details of the microorganisms used for the study.

Grams strain	Name of the organism	Std Code
Gram-negative	*Pseudomonas aeruginosa*	(ATCC-2853)
Gram-negative	*Escherichia coli*	(ATCC-25922)
Gram-positive bacterium	*Bacillus subtilis*	(ATCC-6051)
Gram-positive bacterium	*Staphylococcus aureus*	(ATCC-9144)

Table. 2. Antibacterial zone of inhibition for *Leucas aspera* leaf extract mediated CuO nanoparticles.

Sample	**Zone of Inhibition (mm)**							
	S. aureus		***B. subtilis***		***E. coli***		***P. aeruginosa***	
S1- *Leucas aspera*	500 µg	1000 µg	500 µg	1000 µg	500 µg	1000 µg	500 µg	1000 µg
	10	12	14	16	10	12	10	10
Amikacin (30 µg)	36		36		34		36	

Table 3. Antibacterial zone of inhibition for *Morinda tinctoria* leaf extract mediated CuO nanoparticles.

Sample	**Zone of Inhibition (mm)**							
	S. aureus		***B. subtilis***		***E. coli***		***P. aeruginosa***	
S2-*Morinda tinctoria*	500 µg	1000 µg	500 µg	1000 µg	500 µg	1000 µg	500 µg	1000 µg
	10	14	16	18	12	14	10	12
Amikacin (30 µg)	36		36		34		36	

4. Conclusions

In conclusion, copper oxide nanoparticles were successfully synthesized using the aqueous leaf extracts of *Leucas aspera* and *Morinda tinctoria* plant material, which acted as reducing/stabilizing agents. The structure of the prepared nanoparticles was identified using XRD analysis. The specimen's shape and morphology were studied using SEM instrumentation and found to be spherical and cubical. The strong absorption peaks at 620 cm^{-1} and 615 cm^{-1} confirmed the formation of CuO nanoparticles. The optical band gap was calculated from the results of the UV-Vis spectrum. The prepared CuO nanoparticles' antibacterial activity was tested against a few Gram-negative and Gram-positive bacteria by disc diffusion method. The formed zone of inhibition was compared with the standard values of the reference sample Amikacin. Finally, the resulting green synthesis of CuO nanoparticles indicated appreciable antibacterial activity to find applications in medicine and food packaging.

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Conflicts of Interest

All authors declare that there is no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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