RESEARCH ARTICLE

GREEN SYNTHESIS OF IRON OXIDE NANOPARTICLES FROM LEAF EXTRACT OF PASSIFLORA FOETIDA AND ITS ANTIBACTERIAL ACTIVITY

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ARTICLE INFO	ABSTRACT
	Objective: Synthesis, characterization and antibacterial activity of iron oxide nanoparticles prepared by of *Passiflora foetida* aqueous extract. **Methods:** Iron Oxide nanoparticles (Fe_2O_3 NPs) were synthesized by the reduction of iron chlorides using phyto-chemicals as bio-reductants. The synthesis of Fe_2O_3 nanoparticles was confirmed by UV-Vis spectrophotometry and the average particle size was determined by X-ray diffractometry (XRD). Morphology of the synthesized Fe_2O_3 NPs was identified using Scanning Electron Microscopy (SEM). Further characterization of Fe_2O_3 NPs was done to study its nature by FTIR. Biological activity of Fe_2O_3 nanoparticles was analyzed through its antibacterial effect. **Results:** Synthesized iron oxide nanoparticles exhibit cubic crystalline phase. Morphology was identified as spherical and particle size ranges from 10 to 16 nm in diameter with an average size of 20 nm analyzed from XRD. Fe_2O_3 nanoparticles showed antibacterial activity as that of control drug (streptomycin) towards Gram positive and negative bacteria. Iron oxide nanoparticles were also found to be non-toxic as synthesized by using green synthesis. **Conclusion:** Green synthesis of Fe_2O_3 nanoparticles using *Passiflora foetida* leaves aqueous extrac**t** was considered to be relatively safe and particles possesses significant biological activity.

INTRODUCTION

Multifunctional metal and metal oxide nanoparticles have become an interesting area of research due to its physicochemical properties and potential application ranging from environmental remediation to biomedical uses. Currently, a large number of physical, chemical and biological methods are available to synthesize different types of nanoparticles. Among them, biological method of green synthesis has attracted a lot of attention with the aid of novel, eco-friendly and convenient biological materials namely fungi, bacteria, biomolecules and plants (Michał Moritz, 2013). Hence, nowadays many researchers are diverting themselves from using biosynthetic methods and using plant parts like leaf, fruits, stems and bars extract for synthesis of nanoparticles as it is cost effective and can be easily scaled up to be used for large scale production (Monalisa Pattanayak and Nayak, 2013). Recently, considerable attention has given to exploit the antibacterial properties of leaf (Tayyaba Naseem, 2014; Priya Banerjee *et al.,* 2014) seed (GnanaDhas *et al.,* 2012; Chidambaram Jayaseelan *et al.,* 2013) and other plant parts

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have many medicinal properties against several bacteria, especially gram-negative and positive bacteria (Akinnibosun *et al.,* 2008). The development of new resistant strains of bacteria to current antibiotics has become a serious problem in public health, therefore, there is a strong incentive to develop new bactericides from various sources. Nanotechnology has provided an attractive method for synthesizing alternative antimicrobial agents and toxicity to a wide range of microorganisms and very little is known about the toxicity of iron oxide nanoparticles towards microorganisms (Sudhanshu Shekhar Behera *et al.,* 2012). *Passiflora foetida L. (Passifloraceae),* commonly known as passion fruit, is an exotic fast growing perennial climber occurring in the USA and extended to India. Traditionally, the plant has been used for its properties like antiproliferative, sedative, anti-anxiety, antibacterial, leishmanicidal, antispasmodic, emetic, dressing for wounds and antiulcer (Sudhanshu Shekhar Behera *et al.,* 2012). The present work deals with the green synthesis of iron oxide nanoparticles by using of *Passiflora foetida* leaf extract and characterized by UV-Vis absorption spectroscopy, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and antibacterial activity against five fish pathogenic bacterial strains were used for testing iron oxide nanoparticles and

commercial antibiotic as neomycin (Mohanasundari *et al.,* 2007). *P.foetidia* using iron oxide nanoparticles synthesis is first report in our knowledge of this field.

MATERIALS AND METHODS

The fresh leaf of *Passiflora* was collected from the campus of Gandhigram Rural Institute - Deemed University, Gandhigram, Dindigul, Tamil Nadu, India.

Preparation of plant Extract and the Precursor

Fresh leaves of *P.foetida* were washed with distilled water and chopped into small pieces.10g of chopped leaves were transferred into an Erlenmeyer flask containing 100 ml of distilled water added and maintained at 60 °C for 15 min. The supernatant was filtered through Whatmann No. 1 filter paper to get the leaf extract (pH 6). The extracted material was stored at 4 °C for further studies. The precursor chemicals 0.1 M Ferrous and Ferric chloride (1:2) in 100 ml of distilled water were prepared for iron oxide nanoparticle synthesis.

Phytochemical Analysis

The fourteen phytochemical screening tests were conducted on *P.foetida* leaf extract and results of all the analysis has been tabulated.

Synthesis of iron oxide nanoparticles using *Passiflora foetida* leaf extract

For synthesis, 55ml of leaves extract was taken (pH 6) in 100 ml of Erlenmeyer flask and added 0.1M $FeCl_2$ and $FeCl_3$ (pH 1) in 15 and 30 ml (1:2) respectively and continuously stirred in a magnetic stirrer at 60 ± 1 °C for 30 min. The solution pH of these was adjusted to 11 and the synthesis of nanoparticles was confirmed by color changes from greenish yellow to dark greenish black precipitate. The precipitated solution was centrifuged at 6000 rpm at 5 min. and the pellet was collected, washed with distilled water. The collected pellet was dried at 50°C for 30 minutes and stored in sealed bottles under dry conditions prior to use.

Characterization

The absorbance spectra of samples were measured in wavelength within the range from 200-800 nm using a JASCO-V-530-UV-VIS Spectrophotometer. Structure and crystalline size of nanoparticles were determined by XRD using SHIMADZU (Model XRD¬6000) with nickel-filtered Cu Kα radiation in the 2θ range (λ = 1.5418Å) from an x - ray tube run at 40 kv and 30 ma. The samples were scanned between the angles 0° to 90° to obtain the equatorial reflection. The morphology of the material was studied using Tesco SEM - VEGA III lmu – type of scanning electron microscopy operating at 20kv in the vacuum on powder samples and chemical composition of iron oxide nanoparticles were analyzed by employing Energy Dispersive X-ray Spectrometer. The functional group of leaf extract and nanoparticles were recorded by Thermo-Scientific NICOLETTI IS5 over the spectral range of 400-4000cm^{-1}.

Antibacterial Activity of synthesized Nanoparticles

A total of five fish pathogenic gram-negative (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) and gram-positive

(*Bacillus cereus*, *Staphylococcus aureus* and *E. coli)* bacterial strains were used for the antibacterial study of iron oxide nanoparticles by well-diffusion method. The bacterial culture was grown in nutrient agar media at 37°C for 24 hours. The bacterial culture was spread on nutrient agar plates with the help of L - shaped glass rod for spread out the bacterial culture. Three wells (1. control, 2. synthesized iron nanoparticles, 3. streptomycin) were developed in each plate with the help of sterilized steel borer of 8 mm diameter and 30 L sample suspension was loaded in each well. The plates were incubated for 24 hours at 37°C. The diameter of the inhibition zones was recorded in mm. The experiment was repeated and values were calculated for antibacterial activity.

RESULTS

Phytochemical test performed in the leaf extract of *P. foetida* is presented in Table 1. Out of the fourteen phytochemicals screened, three were present in the leaf extracts of *P.foetidia*. The phytochemicals are present in the leaf extract act as reducing agents, which include Phenolic compounds, Phlobatannins and Tannins. Commonly phenolic and tannins are highly reducing power activity in the iron ions to nanoparticles. From these results primary confirmation is done to synthesis of iron oxide nanoparticles by using plant extract. The UV–visible absorption spectra findings demonstrates a novel technique for the preparation of the iron oxide nanoparticles.
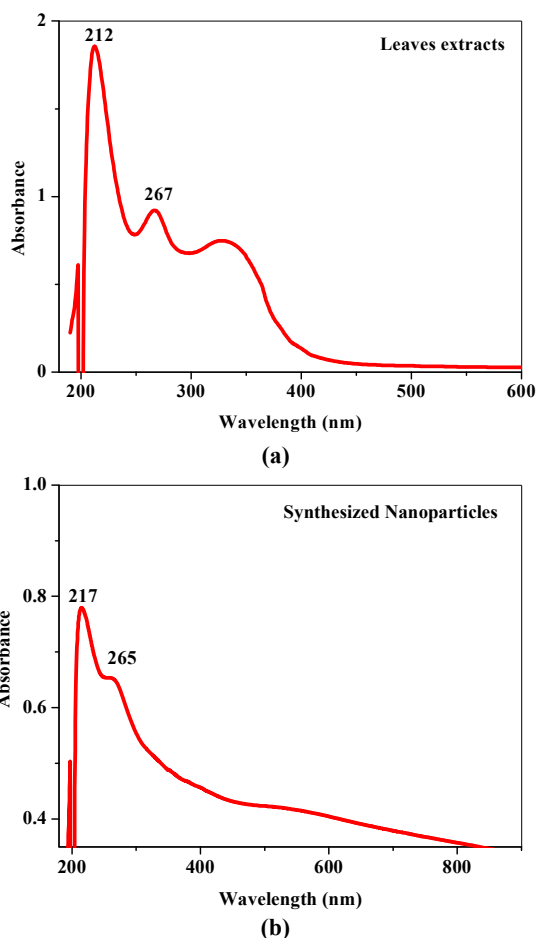

Fig. 1. UV–visible spectral analysis of *Passiflora foetida* leaf extract and synthesized iron oxide nanoparticles

The scale of the wavelength fixes 200-800 nm. The formation of nanoparticles was confirmed by its color changes from

greenish yellow to dark greenish black precipitate. Such colour productions were carried out due to surface plasmon resonance process. The absorbance of leaf extract and synthesized nanoparticles absorbance peaks at 212 nm and its slightly shifted to 217 nm and also with absorbance band present at 265 nm due to capping of oxidized phenolic compounds (Fig.1 [a & b]). It may be confirmed the presence of phenolic compounds in the formation of Fe_2O_3 nanoparticles. This absorbance shows that the leaf extract of *P.foetida* has played as a reducing agent. The phase structure of biosynthesized iron oxide nanoparticles is shown in Figure.2.
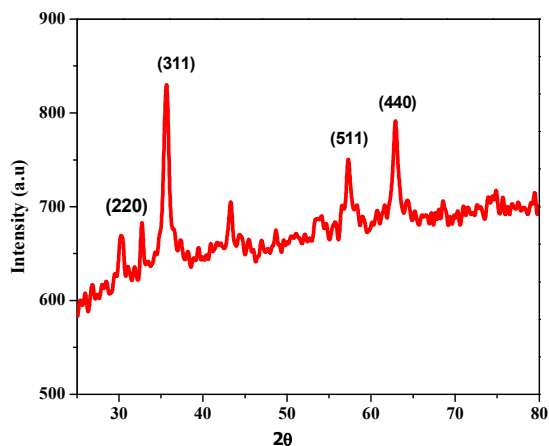

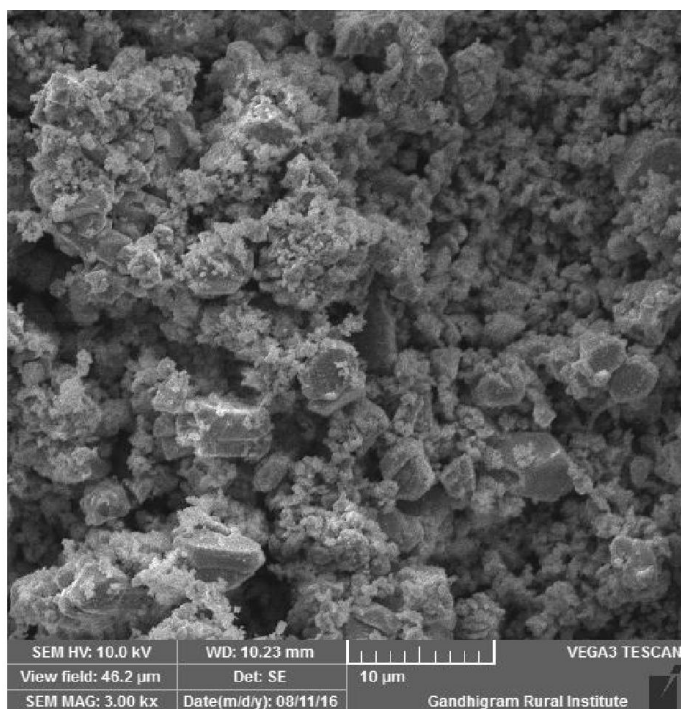**Figure 2. XRD pattern of Iron oxide nanoparticles**

Fig. 3. SEM image of Iron oxide nanoparticles

The 2θ values of 30.20^o, 35.60^o, 57.30^o and 62.80^o in the reference elemental iron (JCPDF No: 083-0112) which are attributed 220, 311, 511 and (440) crystallographic plane of face centered cubic iron crystals. These results indicated that iron oxide nanoparticles are cubic in nature and also supported by the XRD results which indicate that γ-Fe_2O_3 phase contains only Fe^{3+} cations. Furthermore, the approximate crystallite size (D) of the iron oxide nanoparticles was calculated using the Scherer equation and approximate particle size is 16 nm. Scanning Electron Microscopy indicated that nanoparticles

formed as agglomerated because of the adhesive nature of distorted irregular cluster appearance as shown in Figure.3 and due to the denaturation and aggregation during the sputtering process. Elemental composition of synthesized iron oxide nanoparticles using leaf extract of was determined by using EDX analysis. It was observed that the percentage of iron, oxygen, calcium and chloride was 57.88, 29.11, 9.24 and 4.07 % respectively (Figure 4. [a&b]).
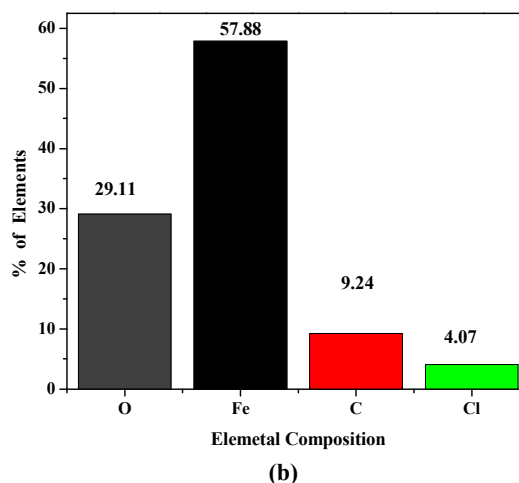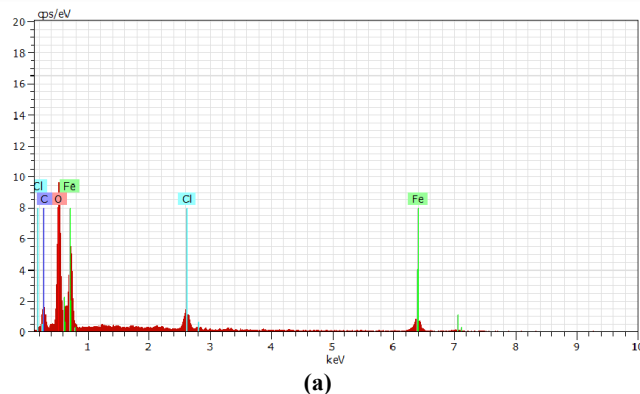

(a)

(b)

Fig. 4. EDX image and Elemental composition of Iron oxide nanoparticles

Calcium and chloride are present in elemental composition, due to the plant constituents are base for synthesized iron oxide nanoparticles. Morphological structure images showed the particles agglomeration to form irregular cluster shape. The FTIR measurement was carried out for identifying the possible bio-chemicals responsible for the formation of Fe_2O_3 iron oxide nanoparticles using leaf extract of *P.foetida*. This characterization was used to identify the functional groups of the bioactive components based on the peak value in the region of infrared radiation. The leaf extract absorbance showed 3395 and 1605 cm^{-1} attributed to the stretching of –NH bonds. The peaks are 2916, 1394 and 619 cm^{-1} denoting the C-H stretch. Also, iron oxide formation were confirmed 3376 and 3155 cm^{-1} bands have –OH stretching and 1633, 1403, 1041 cm^{-1} respectively for the presence of primary amines, aromatics and aliphatic amines. The analysis indicated the absorption peaks at 562 corresponding to the Fe–O vibration related to the magnetite phase. The FTIR result clearly showed that the extracts containing OH as a functional group act in capping the nanoparticles synthesis. Thus, FTIR analysis clearly shows that capping and reducing of nanoparticles by bioactive molecules present in leaf aqueous extract of *P.foetida* could be responsible for prolonged stability (Fig.4 [a & b]).
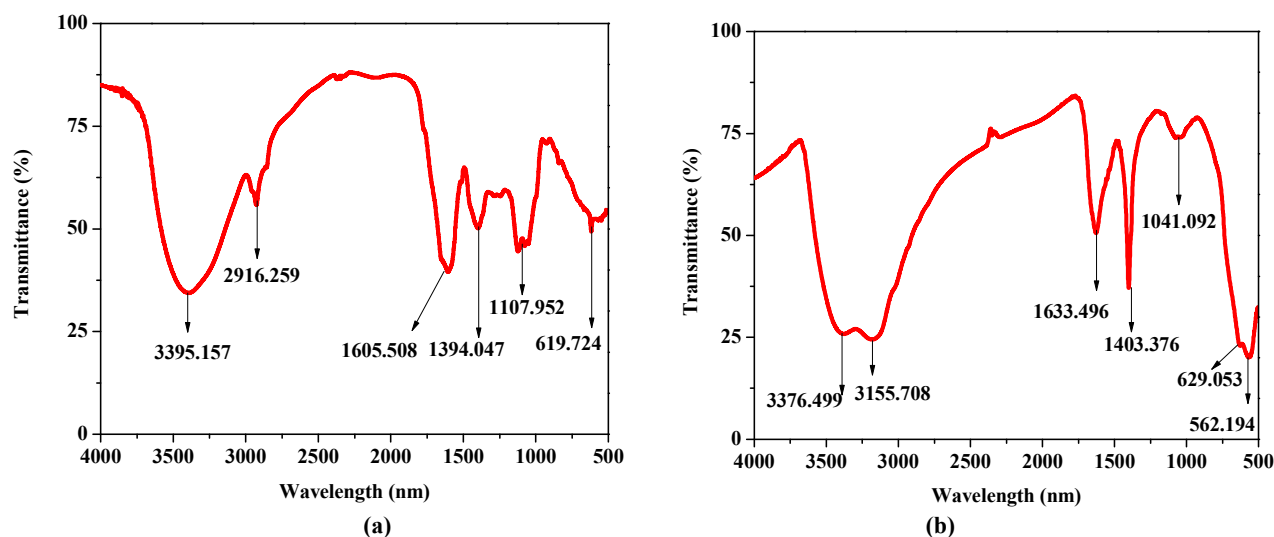
Fig. 5. FT-IR spectroscopy analysis of *Passiflora foetida* leaf extract and Iron oxide nanoparticles

Table 1. Phytochemical tests of the Aqueous leaf extract of *P.foetida*

S.No.	Phytochemicals	Occurrence
1.	Alkaloids	-
s2.	Anthroquinones	-
3.	Amino acids	-
4.	Carbohydrate	-
5.	Flavonoids	-
6.	Glycosides	-
7.	Phenolic compounds	++
8.	Phlobatannins	+
9.	Quinons	-
10.	Saponins	-
11.	Sterols	-
12.	Tannins	+
13.	Terpenoids	-
14.	Reducing Sugars	-

(-) – absence, (+) – Presence, (++) Good

Table 2. Antibacterial activity of iron oxide nanoparticles compare with commercial Antibiotic Streptomycin

	Streptomycin	Metal Precursor	Aqueous extract	Iron oxide nanoparticles
Staphylococcus aureus	23 ± 0.50	20 ± 1.50	12 ± 0.30	20 ± 0.80
Bacillus cereus	30 ± 0.81	19 ± 1.06	14 ± 1.50	23 ± 1.02
Escherichia coli	22 ± 1.06	20 ± 0.62	11 ± 0.05	19 ± 0.91
Klebsiella pneumonia	15 ± 1.02	13 ± 2.05	09 ± 1.72	11 ± 1.05
Pseudomonas aeruginosa	21 ± 1.07	19 ± 0.52	12 ± 0.60	18 ± 0.97

Antibacterial activity of plant extract, chemical, iron oxide nanoparticles and commercial antibiotic is presented in Table 2. Iron oxide nanoparticles synthesized using two medicinal plants and the antibacterial activity against *Escherichia coli, Salmonella enterica, Proteus mirabilis*, and *Staphylococcus aureus* by using well-diffusion method (Tayyaba Naseem, 2014). Iron oxide nanoparticles of *P.foetida* leaf extracts were more resistant to *Bacillus cereus* and the maximum zone of inhibition (ZOI)   was 23 mm as shown in and *Klebsiella pneumonia, Pseudomonas aeruginosa, Staphylococcus aureus* and *Esherichia coli*   the zone of inhibition of iron oxide nanoparticles was 20,19,11 and 18mm respectively. Ferrous and Ferric chloride have good antibacterial activity, because it have naturally toxic in nature. But in nanoparticles stage these ions have high surface activity to detoxify pathogenic bacteria. These results were compared to commercial antibiotic streptomycin and the zone of inhibition was 23, 30, 22, 15 and 21mm. The antibacterial activity against ten human pathogenic bacteria (gram positive and negative).

DISCUSSION

The aqueous *P. foetida* leaf extract are most present phenolic compounds for the   preparation of Fe_2O_3 nanoparticles. Similar phytochemical analysis was also reported in *Datura Inoxia, Senna occidentalis* (L.) and *Centella asiatica* plant extracts in which the phenols, cardiac glycosides, phlobatannins, proteins and tannins were present in leaves extract (Amlan Kumar Das *et al.,* 2014; Oluwakayode Odeja *et al.,* 2015; Lakshmi Pravallika *et al.,* 2015). Bioreduction of Fe^+ through conforming through the UV-visible spectrum absorption peaks at 216-268 nm regions. It may confirm the bioreduction process of mixture the plant extracts and precursor chemicals (Monalisa Pattanayak, 2013). The absorbance of leaf extract peak at 214 nm and peak was slight shifted at 260 nm confirmed the presence of phenol compounds and formation of iron oxide nanoparticles may due to the presence polyphenols complex present at 290 nm (Harshiny Muthukumar *et al.,* 2015). Iron oxide nanoparticles was synthesized using *Eucalyptus globule* leaf extract and its XRD characterization   showed the results as ß Fe_2O_3 to

conforming JCPDS card number (Matheswaran *et al.,* 2014). γ-Fe_2O_3 particle size was calculated using the Scherer equation estimated at around 7.7 nm (Carmen Steluta Ciobanu *et al.,* 2012). Elemental composition of iron oxide nanoparticles were Iron, Oxygen, Calcium and Chloride also present (Eric *et al.,* 2011). Iron oxide nanoparticles were more aggregates to formed post synthesis. This agglomeration can be avoiding by use of the calcinations process to get clear morphological structure and particles shape (Narendhar Chandrasekar *et al.,* 2013). The Fe–O stretching was established by vibration at 566 cm^{-1} based on compound stretching to shows the formation of iron oxide nanoparticles. This peak has been confirmed the iron ions reducing the nano level particles by using plant extract (Arokiyaraj *et al.,* 2013). Same results are reported the formation of Fe_3O_4 and characterized by two absorption bands at 535 and 307 cm−1 which correspond to the Fe–O bond (Mahnaz Mahdavi *et al.,* 2013).

Conclusion

From the present study, it is inferred that the green synthesis of iron oxide nanoparticles using leaf extracts of *P.foetida* containing rich amount of phenolic compounds were found to be very effective, reducing and stabilizing by forming nanoparticle based on phytochemical results. The aqueous leaf extract of *P.foetida* appears to be environmental friendly, so this protocol could be used for rapid production of Fe_2O_3 nanoparticles.   In future, selection of such plants may create a new platform for realizing the potential of herbal medicines in nanoscience. Furthermore, it shows better antibactericidal activity in gram-positive and negative bacteria as compared to commercial antibiotic streptomycin. The present study highlights the potential application of iron oxide nanoparticles as antibacterial agents which can be explored for its typical application in pharmaceutical and biomedical industries and opens the path for further research on the toxicity and carcinogenicity properties.

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