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BIOLOGICAL EVALUATION AND GREEN SYNTHESIS OF SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *CALOTROPIS PROCERA*

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ABSTRACT

The unique property of the silver nanoparticles having the antimicrobial activity gained the major attention towards the present nanotechnology. Green synthesis of silver nanoparticles (AgNPs) was performed from aqueous silver nitrate using *Calotropis procera* leaves (L) and stem (S) extract. The synthesized silver nanoparticles have been characterized by the UV-visible spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM). The XRD analysis shows that the AgNPs are of face centered cubic (FCC) structure and size was ranging from 19 to 45 nm (L) and 26 to 38 nm (S) respectively. Scanning electron microscopy (SEM) analysis showed that the synthesized AgNPs have spherical shape. The silver nanoparticles possess significant antimicrobial potential against *K. pneumonia* and *S. typhi* and also showed good antioxidant property.

KEYWORDS: ***Calotropis procera*, silver nanoparticles, XRD, antimicrobial activity, antioxidant activity.**

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INTRODUCTION

The nanotechnology is an important branch in the major fields of biology, chemistry, physics, and material sciences. Nanotechnology deals with the study of materials at the nanometers[1,2]. The nanomaterials can be synthesized by different methods including chemical, physical, irradiation, and biological methods. The development of new chemical or physical methods has resulted in environmental contaminations, since the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts[3]. Thus, there is a need for "green synthesis" that includes a clean, safe, ecofriendly, and environmentally nontoxic method of nanoparticle synthesis and in this method there is no need to use high pressure, energy, temperature, and toxic chemicals[4,5]. Nanoparticles possess a wide array of application in the different field's *viz.* medicine, electronics, therapeutics, and as diagnostic agents. Silver nanoparticles have wide application in biomedical sciences like treatment of burned patients, antimicrobial potential against various pathogenic microorganisms and used the targeted drug delivery[6]. Silver nanoparticles are reported to possess anti-fungal, anti-inflammatory, anti-viral, anti-angiogenesis and antiplatelet activity[7,8,9]. Nowadays the nanoparticles are coated on the medical appliances, food covering sheets, and cans for storing the beverages and food[10,11,12]. However, there are many problems and toxicity of using metal oxide nanoparticles on the human health. Use of plants for the synthesis of nanoparticles does not require high energy, temperatures, and it is easily scaled up for large scale synthesis, and it is cost effective too[13,14,15].

C. procera is a wild plant (family *Asclepiadaceae),* well known for its medicinal properties. It has been known to possess analgesic, antitumor, antihelmintic, antioxidant, hepatoprotective, inflammatory, antidiarrhoeal, anticonvulsant, antimicrobial, oestrogenic, antinociceptive, and antimalarial activity[16,17]. *C. procera,* is a plant with good enough quantities of latex i.e. milky liquid, when any mechanical damages, their tissues are broken and secrete the milky latex. It contain several biologically active compounds, including proteins, amino acids, carbohydrates, lipids, vitamins, alkaloids, resins, and tannins[18]. The green synthesis of AgNPs using flower extract of *C. procera* and leaf extract of *C. gigantea* has been reported[19,20]. The present communication reports green synthesis of AgNPs using leaf and stem extract of *C. procera*. To the best of our knowledge, biological approach using leaves and stem of *C. procera* has been used for the first time as a reducing material as well as surface stabilizing agent for the synthesis of spherical-shaped AgNPs. The structure, phase, and morphology of synthesized product were investigated by the standard characterization techniques.

MATERIALS AND METHODS

2.1. Materials

All analytical reagents and media components were purchased from Merck Chemicals. The deionized water was used throughout the experiment. All glassware's were properly washed with distilled water and oven dried before use. Fresh leaves and stem of *C. procera* were collected from Srinagar Garhwal, Uttarakhand and identified by Department of Botany, HNB Garhwal University, Srinagar (Garhwal), Uttarakhand.

2.2. Preparation of Plant extract

The fresh plant material of *C. procera* was collected and shade dried at room temperature for about ten days. The dried plant material was powdered and 10 g of powdered material boiled with 100 mL of double distilled water for 5 minutes in the water bath. The solution was cooled at room temperature and filtered by Whatman filter paper No. 1. The filtrate was collected and stored at 4°C for further experiment.

2.3 Preparation of silver nanoparticles

Silver nanoparticles were synthesized by reducing the freshly prepared 1 mM silver nitrate ($AgNO_3$) and stored under dark conditions with aqueous extract of the plant.

The reaction mixture was prepared in ratio of 9: 1 (V/V) comprised of freshly prepared silver nitrate solution and plant extract respectively. The initial color of the solution was observed. The solution was stored at room temperature for 24 hours for the complete settlement of nanoparticles. After 24 hours the reaction mixture was centrifuged and pellets were collected followed by washing with deionized water and dried in water glass.

2.4. UV-VIS spectra analysis

The silver nanoparticles show the plasmon resonance at 400 to 450 nm in the UV-Visible spectrum. The UV-Visible spectrum of synthesized silver nanoparticles was analysed by spectrophotometer (UV-Visible Perkin Elmer Lambda 25).

2.5. SEM analysis of silver nanoparticles

SEM analysis of the silver nanoparticles provides the information regarding the dimensions including the surface, shape, and size. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid and observed under EVO 50 SEM. Extra solution was removed using a blotting paper and then on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min.

2.6. X-ray diffraction analysis of silver nanoparticles

The particle size and nature of the AgNPs were determined using XRD PW3040/60 X-pert PRO (Netherlands), operating at a voltage of 45 kV, a current of 40 mA with Cu K α radiation at 2θ angle ranging from 5˚ to 90˚. The particle size of the particles on the silver nanoparticles was determined using Debye Sherrer's equation.

$$D = 0.94 \lambda / \beta \cos \Theta$$

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle.

2.7. Antibacterial Property of AgNPs

The antibacterial property of the silver nanoparticles was determined against the pathogenic bacteria *viz. Staphylococcus aureus, Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumonia* by disk diffusion method[21]. The different concentrations of AgNPs were used in DMSO viz., 100% (10 µg/ml), 75% (7.5 µg/ml) and 50% (5.0 µg/ml), for the identification of antimicrobial activity. All the plates were incubated at 37°C for 24 hours, and the zone of inhibition of bacteria was measured.

2.8. Antioxidant activity of AgNPs

The antioxidant activity of AgNPs were determined by free radical scavenging activity i.e. DPPH method[22]. AgNPs of *C. procera* dispersed in methanol (10–100 µg/ml) were added to different test tubes and the volume was made up to 4 ml using methanol. Then, 3 ml of DPPH (0.1mM) solution was added and incubated in dark room for about 30 min at room temperature. The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm against a blank with an ultraviolet-visible spectrophotometer (Perkin Elmer Lambda 25). Gallic acid was used as positive control. The ability to scavenge the free radical, $DPPH^{\bullet}$ in percent (%) was calculated using the following formula

$$\% \text{ of radical scavenging activity} = [A \text{ control} - A \text{ sample} / A \text{ control}] \times 100$$

Where A control is the absorbance of the control sample (DPPH solution without test sample) and A test is the absorbance of the test sample (DPPH solution and test sample).

RESULTS AND DISCUSSION

3.1 UV-Vis spectra Analysis

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles (Sun et al., 2001).The green synthesis of silver nanoparticles using *C. procera* extract was successfully carried out. The change in the colour of the solution from yellowish brown to dark brown exhibited the

reduction of the silver nitrate in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[23]. The formation of silver nanoparticles was confirmed through measurement of UV-Visible spectrum of the reaction mixture. The UV-Visible spectrophotometric analysis of colloidal reaction mixture of silver nanoparticles synthesized using leaf and stem extract of *C. procera* showed peak at 449.34 nm and 452.6 nm respectively, in the spectrum, and broadening of peak indicated that the particles are polydispersed[24] (Figure 1).

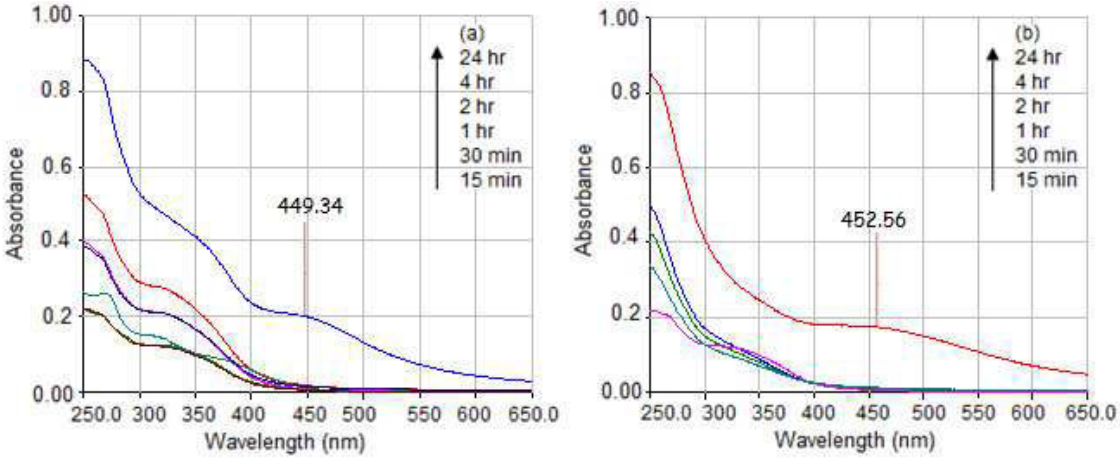

Figure 1
UV-Vis absorption spectra of AgNPs (a) L; (b) S

3.2 SEM analysis

Scanning electron microscopy provided further insight into the morphology and size details of the silver nanoparticles. The SEM image showing the high density silver nanoparticles synthesized by the *C. procera* extract further confirmed the development of silver nanostructures. (Figure 2)

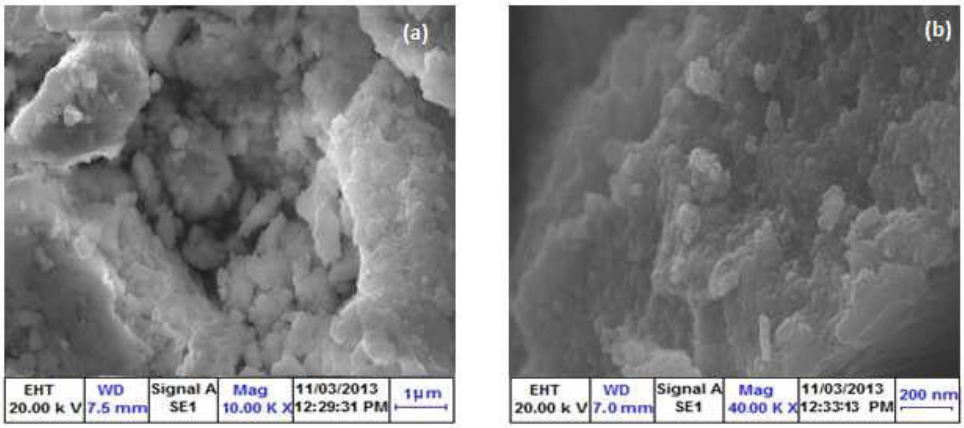

Figure 2
SEM micrograph of AgNPs (a) L; (b) S

3.3 XRD analysis

The XRD pattern of synthesised silver nanoparticles using *C. procera* extract were recorded and typical XRD pattern is shown in Figure 3. The diffraction peaks are indexed as (111), (200), (220), (311) and (222) planes of a pure face centred crystalline (fcc) structure of silver. Crystallite size of AgNPs as estimated from the FWHM of different peaks using the Scherrer's formula and diffraction

lines observed at 2θ angle are given below (Table.1). Apart from these peaks, the recorded XRD pattern shows additional unassigned peaks. This may be due to the formation of the crystalline bio-organic compounds/metalloproteins that are present in the *C. procera*. The present study is in consistence with Shivshankar *et al*., where silver nanoparticles were synthesized using *P. graveolens* leaf broth[25].

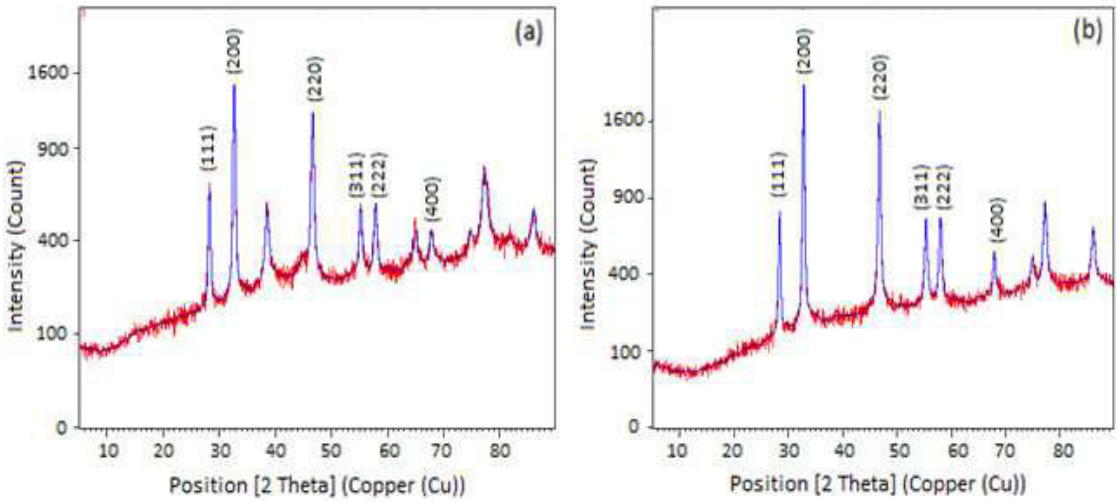

Figure 3
XRD pattern of silver nanoparticles of C. procera (a) L; (b) S

Plant extract	2θ value (degree)	d- spacing (Å)	FWHM (degree)	Plane	Average Particle size (nm)
Leaf	28.25	3.15614	0.4475	111	33.37
	32.66	2.73914	0.4759	200	31.07
	46.66	1.94505	0.5554	220	28.39
	55.25	1.66126	0.5726	311	28.21
Stem	28.38	3.14211	0.3836	111	38.93
	32.80	2.72801	0.3875	200	38.95
	46.78	1.94030	0.4901	220	32.19
	55.37	1.65791	0.5586	311	29.27

Table 1
Crystalline size of AgNPs synthesized using C.procera L and S extract

3.4 Antimicrobial activity of AgNPs

The antimicrobial activity of AgNPs and standard were tested by disk diffusion method. It was found to be concentration dependent (100% > 75% > 50%). The zone of inhibition increased with increased concentration of AgNPs[26]. Synthesized silver nanoparticles showed antibacterial activity against all the four bacteria. The highest zone of inhibition was observed for *K. Pneumonia* and *S.typhi* and found to be less effective against both *E. coli* and *S. aureus*. (Figure 4 & Table 2)

Bacteria	Zone of inhibition (mm)								
	AgNPs of Leaf			AgNPs of Stem			Ciprofloxacin (std.)	Gentamycin (std.)	Control 10% DMSO
	100%	75%	50%	100%	75%	50%			
S. aureus	11	10	8	10	9	6	15	19	-
E. coli	12	9	7	11	10	9	20	20	-
S. typhi	14	12	10	12	7	5	21	18	-
K. pneumonia	20	10	10	15	12	10	19	21	-

Table 2
Antibacterial activity of AgNPs of C. procera by disk diffusion method

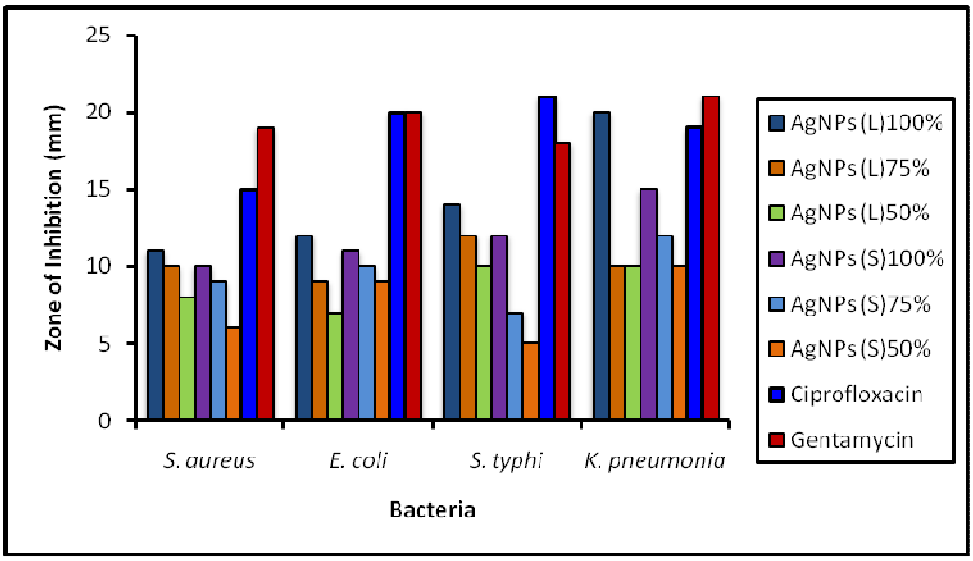

Figure 4
Antibacterial activity of AgNPs of C. procera by disk diffusion method

3.5 Antioxidant activity of AgNPs

DPPH is a stable free radical and shows a characteristic absorption at 517 nm, whose color changes from violet to yellow upon reduction[27]. The antioxidants react with DPPH and convert it to 1,1-diphenyl-2-picryl hydrazine with decolorisation. As shown in Fig. 6, the AgNPs of leaf have higher antioxidant property compared to stem AgNPs but both the extracts exhibited lower antioxidant property compared to gallic acid. The antioxidant activity of AgNPs method is based on the electron transfer reaction between silver and 1,1'-diphenyl-2-picryl hydrazyl radical (Figure 5). Here AgNPs quenched the activity of DPPH by donating their electrons. It has been reported that the antioxidant activity of *M. uniflorum* is a result of phenolic acids.

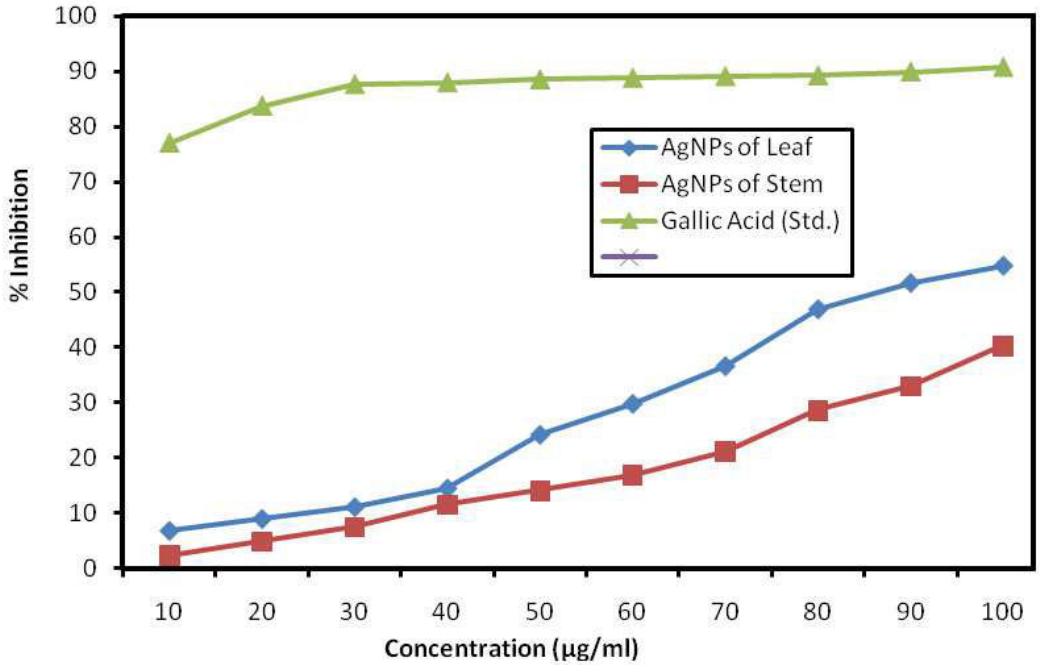

Figure 5
Antioxidant property of AgNPs compared with std. gallic acid

3.6. Mechanism of the formation of silver nanoparticles

Several factors influence the formation of silver nanoparticles such as plant source, organic compound in plant extract. Organic compounds like alkaloids, polyphenols, proteins and even some pigments are present in plant extracts. The phytochemical analysis of *C. procera* has indicated the presence of phenolic acids like o-pyrocatechuic acid, isorhamnetin 3-O-robioside, 3,3',4',5,7-pentahydroxyflavone 3-rutinoside, isorhamnetin 3-β-O-rutinoside etc.[18] (Figure 6). The phenolic acids are reported to be powerful antioxidants. It has been reported that they posses hydroxyl and carbonyl groups which are able to bind to metals[28]. In the present work, the reduction was found to be enhanced on using aqueous extract of *C.procera*. This active hydrogen may be responsible for the reduction of silver ions leading to the formation of silver nanoparticles.

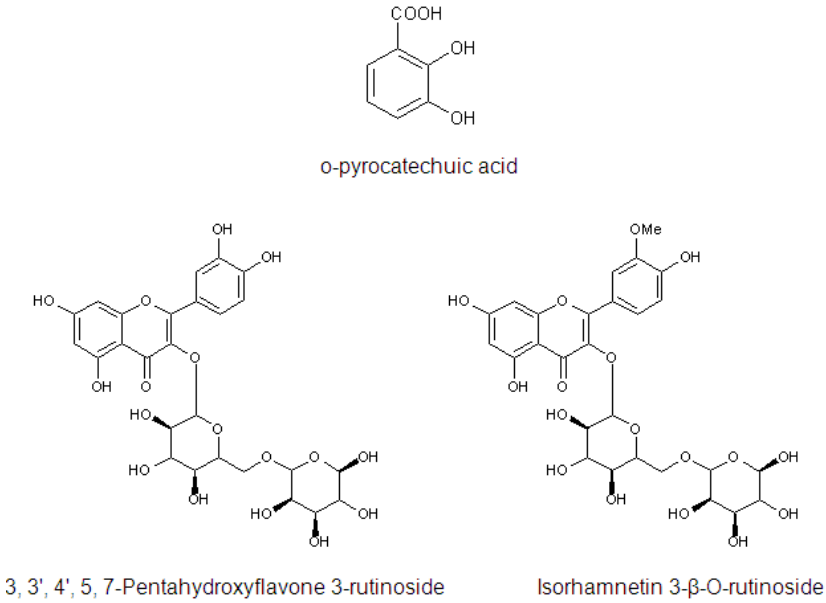

Figure 6
Structure of compounds isolated from C. procera

CONCULSION

The present communication reports the efficiency of leaf and stem extract of *C. procera* for ecofriendly bioreduction of $AgNO_3$ into silver nanoparticles. The nanoparticles were characterized by UV–Vis, SEM and XRD measurements. These nanoparticles were crystalline in nature and were stable upto 30 days. The synthesized silver nanoparticles are stable due to the presence of proteins acting as capping and reducing agents. The results showed that Ag nanoparticles presented remarkable antimicrobial activity against common pathogens. It can be concluded that the silver nanoparticles constitute an effective antimicrobial agent against common pathogenic microorganisms. Biologically synthesized silver nanoparticles could be of immense use in medical textiles for their efficient antibacterial and antimicrobial properties[29]. In addition, AgNPs possess significant antioxidant property. Green synthesized AgNPs have various applications in biochemical–pharmacological investigations such as antimicrobial, antioxidant, anticancer and wound healing activities.

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