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Green Synthesis of Silver Nano particles Using Leaves Extract of Euphorbia Heterophylla and Its Antibactrial Activity

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Abstract - The Article reviews synthesis of Silver nanoparticles using Euphorbia Heteropylla leaves extract. The Silver nanoparticles were characterized using the following methods. Bio-synthesized Silver nanoparticles were primarily confirmed by change in colour from yellowish brown to brown. Ultraviolet-Visible Spectroscopy of the Silver nanoparticles showed Absorbance and Transmittance. Fourier Transform Infrared Spectroscopy was used to key out the specific functional groups present in the leaves extract. The X-ray powder diffraction the structural phase of the Silver nanoparticles was found in the form of face centered cubic (FCC). Field Emission Scanning Electron Microscopy analyses revealed that the synthesized Silver nanoparticles were shape and size was confirmed and the Energy Dispersive X-ray analysis spectrum showed the presence of elemental compound peaks in the range 0 keV to 10 keV. The synthesized nanoparticles were analyzed o know the average size 125 nm were confirmed by Dynamic Light Scattering. The two gram positive and one gram negative bacterial strains such as Staphylococcus epidermis, Bacills subtilis and Klebsiella pneumonia were experienced to analyse the antibacterial activity of the Silver nanoparticles.

Keywords: Euphorbia Heteropylla, Antibacterial activity, XRD, FESEM, EDX, FTIR, UV, DLS and Silver nanoparticles.

I. INTRODUCTION

Nanomaterials are foundations of nanoscience and nanotechnology. Nanostructure science and innovation is a wide and interdisciplinary zone of innovative work action that has been becoming dangerously worldwide in the previous ten years. It has the potential for reforming the manners by which materials and items are made and the reach and nature of functionalities that can be gotten. It is now having a critical business sway, which will definitely increment later on [1-4]. Nanoscale materials are characterized as a lot of substances where in any event one measurement is not exactly around 100 nanometers [5]. Nanomaterials are of intrigue in light of the fact that at this scale one of a kind optical, attractive, electrical, and different properties develop. These eminent properties have the potential for incredible effects in hardware, medication, and different fields. There are numerous synthetic, physical, and organic green union techniques to set up the nanoparticle shape and size for the previously mentioned wanted applications [6-8]. The current work is an endeavor to orchestrate Silver compound precipitation strategy utilizing plant remove in watery arrangement. Recently, research on composite materials made the possibility of misusing size-subordinate belongings opens thusly the path toward the improvement of new utilitarian materials and progressed gadgets. Nano-particles normally alluded as particles with a size up to 100 nm [9-11]. Nano-particles show totally new or improved belongings dependent on explicit attributes, for example, size, appropriation and morphology, whenever contrasted and bigger particles of the mass material they are made of Nano-particles present a higher shallow to volume proportion with diminishing size of nano-particles. In this investigation, we screened Euphorbia Heteropylla plant leaf extricate and thought about their blend of silver nano-particles by checking the characterize of UV-visible spectroscopy, optical discharge of FT-IR, structure decide of XRD, morphology imaging of FESEM, presence of components in EDX, size dissemination of DLS and their organic portray of antibacterial exercises [12-18]. The antibacterial activites of the samples were studied on Staphylococcus epidermidis (MTCC 737), Bacillus subtilis (MTCC 2451) and Klebsiella pneumonia (MTCC 3384) [19].

II. EXPERIMENTAL

Materials Silver Nitrate (Analytical evaluation) was bought from Sigma-Aldrich Chemical Pvt. Ltd. All the watery arrangements were readied utilizing twofold refined de-ionized water. 5 mM (0.08493 g) of silver nitrate was weakening in 500 ml water underneath continued blending for 1 hour to get $AgNO_3$ fluid arrangement.

Figure 1 Euphorbia Heteropylla

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Assortment of Plant Material

Figure 1 shows the Euphorbia heteropylla leaves were gathered from viriyur village kallakurichi district in Tamil Nadu state. The gathered leaf was firmly gathered with Polyethene pack and afterward moves to the research center. At that point it was washed a few times under running water and afterward washed with refined water twice to eliminate dust particles and held under room temperature for about fourteen days in dull condition. At that point it was make into powder utilizing blender.

Planning of Plant Extract

The powder of Euphorbia heteropylla leaf was weighed 2g and disintegrated in 40ml of twofold refined water and warmed to 50˚C with nonstop mixing time for 30 minutes. The blends were cooled to get temperature, separated with whatmann No.1 channel paper to get the concentrate (100%). The Leaf extract was gathered and put away in test bottle. The concentrate capacities as both the diminishing and balancing out specialist [20].

Synthesis of Silver nanoparticles utilizing Euphorbia heteropylla leaf extract

A 80 ml of the fluid arrangement was readied which comprises of 1mM Silver nitrate is (AgNPs) and this arrangement is utilized for the blend of silver nanoparticles. To accomplish a productive amalgamation, 8 ml of Euphorbia heteropylla leaf separate was included into 80 ml fluid arrangement of silver nitrate with consistent blending on an attractive stirrer for 1-hour and under kept the room temperature 24 hour in dim condition. The shading change of the arrangement was checked. At that point the examples were at consistent 8000 rpm, the completely dense arrangement was centrifuged for around 10 minutes. The total breakdown of the metal particles was gotten. The incorporated silver nanoparticles were gathered and put away in test bottle. The earthy colored shade of arranged nanoparticles from dull demonstrates 100% transformation of silver nanoparticles as appeared in the figure 2.

Fig 2 Color change of plant extract after the addition of $AgNO_3$

III. CHARACTERIZATION

The formation of silver nanoparticles proved by UV-Visible spectroscopy using UV-VISIBLE SPECTROPHOTOMETER LAMBDA 365 PERKIN ELMER showing ranges between 190 nm to 1100 nm. FTIR analysis was accomplished for the reduction of silver ions with the spectral range of 400-4000 cm-1using FTIR SPECTRUM RX I PERKIN ELMER SPECTROMETER instrument. The silver nanoparticles having crystalline structure was determined by the XRD examples of chose examples exist recorded use (XPERT-PRO) diffract meter. Anode material Cu, K-alpha frequency 1.54060 Å, was utilized to record the example at 25°C of estimation temperature. To examine the morphology of silver nanoparticles by employing Field Emission Scanning Electron Microscopy by using the sample exist recorded use (make and model) FESEM : CARL ZEISS (USA), SIGMA WITH GEMINI COLUMN, Resolution 1.5 nm, In focal point Detector, SE2 Detector, BSD Detector. with an Energy Dispersive X-ray Spectroscopy the basic examination (Point check, Area Scan, Line Scan and Elemental Mapping) of the sample affirmed by EDX (make and model) EDAX MAKE : BRUKER (GERMAN) , MODEL :Nano XFlash Detector. Dynamic Light Scattering analysis were used to determine the size of the silver particles by the help of instrument by (DLS) molecule size analyzer (make and model) MICROMERITICS Nanoplus.

Antibacterial activity

Collection of test organisms:

To examine the antibacterial activity of Euphorbia Heteropylla silver nanoparticles, two gram positive bacterial strains Staphylococcus epidermidis (MTCC 737) and Bacillus subtilis (MTCC 2451). One gram negative bacterial strains Klebsiella pneumonia (MTCC 3384) were prepared as test organisms. All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37ºC and maintained on nutrient agar (Difco, USA) slant at for 4ºC.

Screening of Antibacterial Activities (disc diffusion method):

Antibacterial activity of Euphorbia Heteropylla silver nanoparticles (disc diffusion method) was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Muller Hinton Agar and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of various samples respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. Positive control was prepared using the 10 µl of Amoxicillin as standard antibiotic disc. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters and the experiment was repeated twice.

IV. RESULTS AND DISCUSSION

Optical Analysis of UV-Visible absorption Spectroscopy

(a) UV-Visible absorption spectrum

Absorption spectroscopy is a robust non-destructive technique to explore the optical properties of silver nanoparticles. The UV-Visible absorption spectrum of the colloidal $AgNO_3$ nanoparticles has been carried out using UV-Visible spectrometer in the wavelength range 200nm to 1100nm at room temperature shown in figure 3(a). The absorption peak is observed at wavelength 437 nm which clearly indicates the blue shift of synthesized AgNPs using Euphorbia heteropylla sample. During the synthesis, the colour of suspension has changed from yellowish brown to
brown which shows the formation of nano sized silver metals and their given table 1 shows the peak values.

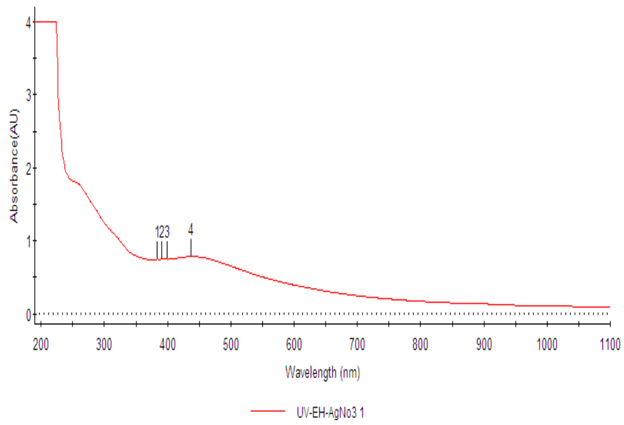


Fig 3(a) UV-Visible absorbance spectrum of Ag NPs using Euphorbia heteropylla

Table 1: UV-Visible absorbance spectrum

S.No	Peak (nm) Wavelength	Peak (AU) Absorbance
1	383.85	0.7476
2	391.30	0.7519
3	398.75	0.7556
4	437.20	0.7858

(b) UV-Visible transmission spectrum

Figure 3 (b) shows that UV-Visible transmission spectrum in the wavelength range 200nm to 11000nm at room temperature. The transmission peak is observed at wavelength 437 nm which clearly indicates the blue shift of synthesized AgNPs using Euphorbia heteropylla sample and their given table 2 shows the peak values.

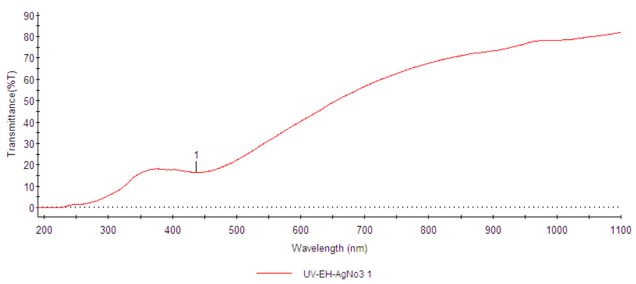


Fig 3(b) UV-Visible Transmittance spectrum of Ag NPs using Euphorbia heteropylla

Table 2: UV-Visible Transmittance spectrum

S.No	Peak (nm) Wavelength	Peak (% T) Transmittance
1	437.20	16.3766

Optical Emission of FT-IR Analysis

Figure 4 shows the FT-IR spectrum of AgNPs of Euphorbia heteropylla in the region 400-4000 cm^{-1}. The vibration frequencies of the various chemical bonds in the nanoparticles can be assigned from the spectrum in terms of band position are given table. The sample can be identified by the assignments of stretching and bending modes of vibration frequencies.

The spectrum showed the nine major bands at 3401 cm^{-1}, 2927 cm^{-1}, 1616 cm^{-1}, 1606 cm^{-1},1384 cm^{-1}, 1270 cm^{-1}, 1075 cm^{-1}, 620 cm^{-1}, and 536cm^{-1}. The spectrum indicates a strong broad band at 3401 cm^{-1} is attributed to O – H stretching vibration. While the broad peak at 2927 cm^{-1} belongs to O – H unit of –COOH group of carboxylic acid. A small peak at 1616 cm^{-1} specify the C = C stretching vibration of alkenes compound and 1606 cm^{-1} wave number is N – H group of bending vibration of amine and the peak at 1384 cm^{-1} C - H group of bending vibration of aldehyde compound. The peaks appeared at 1270 cm^{-1} and 1075 cm^{-1} are C – O group of stretching vibration compound of alkyl aryl ether. The wave numbers 620 cm^{-1} and 536 cm^{-1} are related to C – Br stretching vibration and C – I stretching vibration group of halo compound strong intensity. In these below given table 3, which have described at wavelength, vibration, compound class, appearance and which functional group presented are analyzed.

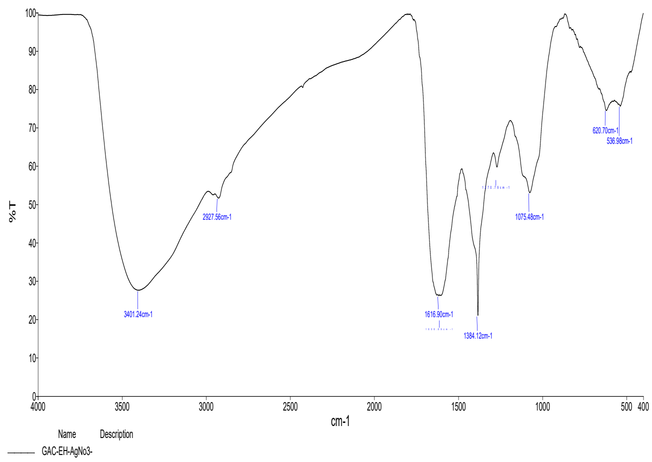


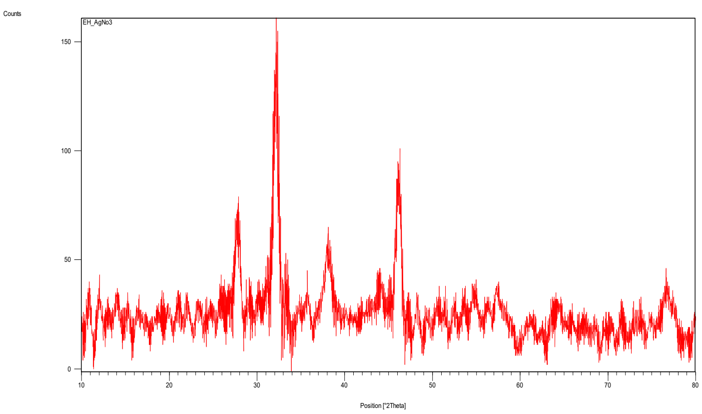
Figure 4 FT-IR Spectrum of AgNPs using Euphorbia heteropylla

Table 3: FT-IR Spectrum Values

Wave number peaks (cm^{-1})	Functional group	Type of vibration	Compound Class	Appearance
3401	O – H	Stretching	Alcohols	Strong, broad
2927	O – H	Stretching	Carboxylic acid	Strong, broad
1616	C = C	Stretching	Alkenes	Strong
1606	N – H	Bending	Amine	Medium
1384	C – H	Bending	Aldehyde	Medium
1270	C – O	Stretching	Alkyl aryl ether	Strong
1075	C – O	Stretching	Alkyl aryl ether	Strong
620	C – Br	Stretching	Halo compound	Strong
536	C – I	Stretching	Halo compound	Strong

Structural Investigation of XRD

In this work, the X-ray diffraction pattern of the prepared Silver nanoparticles is recorded using the XPERT-PRO diffractometer system. Cu K_{α} line with wavelength of 1.5406 Å is generated with a setting of 30 milli amperes and 40 kV with the electrode. The diffracting angle is scanned from 10.00 degrees to 80.00 degrees with a step size of 0.02 degrees. The whole process takes place at a temperature of 25°C. The XRD profile of AgNPs of Euphorbia heteropylla prepared at room temperature is shown in Fig.5. From the figure the diffraction peak indicated at $2\theta=27.62^0$, 32.10^0, 38.12^0, 46.08^0, and 77.04^0 corresponding indicies to (111), (200), (220) and (311) planes of the metallic silver nanoparticles with a face-centered cubic (FCC) crystal structure. From the figure obtained spectrum the corresponding values of 2θ and their intensities for the peaks are noted the Joint Committee on Powder Diffraction Standards (**JCPDS**) files to identify the crystal structure and small particles size of the as-synthesized silver nanopartices. The figure 5 show the peak at 32.10 pertaining to (111) diffraction peak specify the presence of the pure silver and the euphorbia heteropylla capped layer on the silver nanoparticles prevents silver particles from oxidizing. No



diffraction peaks pertaining to other impurities are detected. In addition, the background noise is attributed to the euphorbia heteropylla extract adsorbed the silver nanoparticles .This can be explained that, because of the surface adsorption of euphorbia heteropylla extract, the silver nanoparticles maintain good oxidation resistance. The (111) specify the resultant diffraction peak indicate that the products consisted of pure aspects [21-22].

Fig 5 XRD analyses of AgNPs using Euphorbia heteropylla leaf extract

Field Emission Scanning Electron Microscopy Analysis

The surface morphology of AgNPs using euphorbia heteropylla was investigated that FESEM. The micrograph observations showed synthesize nanoparticles was not in direct contact even within the aggregates, indicating stabilization of the AgNPs. The synthesized nanoparticles were found to be predominantly spherical, round, and even shapes. The morphological shapes and size of the aggregations were described in terms of the fractal dimensions and box-counting method. The result indicates the reduction process being held in the surface by representative FESEM micrograph. The Figure 6.(a), (b), (c) & (d) show in morphology with sizes ranging from 100 nm, 200 nm, 1μm, and 2μm under the various microscopic analyses.

Energy Dispersive X-ray Spectrometer Analyses

The composition of the Silver nanoparticles is calculated using (EDX) elemental of point scan, area scan, line scan and elemental mapping. The elemental composition of green synthesis prepared silver nanoparticles was confirmed by EDS. The Figure 7 reveals that the results of EDX analysis. In this result determined the elemental peaks 0 Kev to 10 Kev and their strong elemental peak at around 2.6 kev to 3.2 kev is found which is in congruence with the major emission peaks specified for metallic silver, indicating the formation of silver nano particles.
This result is consistent with the literature values. Along with this, small peak of oxygen and carbon were also observed because of the capping of silver nanoparticles with bio-molecules of Euphorbia heteropylla leaf extract. Apart from these peaks, no other peak can be formation of pure silver nanoparticles [23].

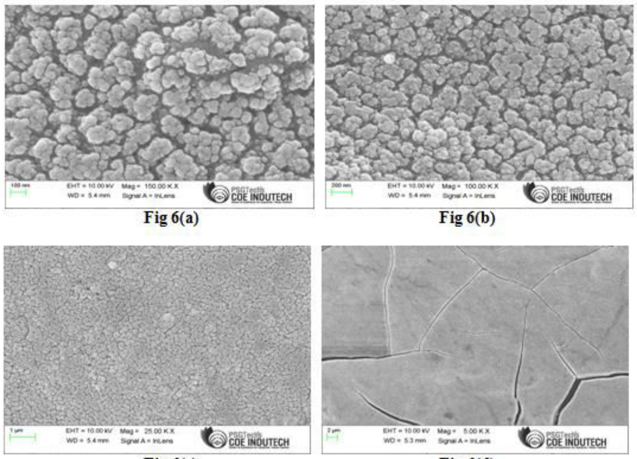


Fig 6(a) Fig 6(b)

Fig 6(c) Fig 6(d)

Figure 6 FESEM images of EH-AgNPs samples

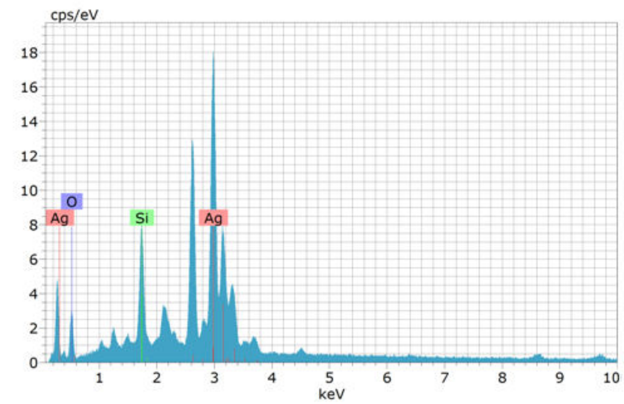


Figure 7 EDX Spectrum of Silver nanoparticles capping of EH extract

Dynamic Light Scattering Analysis

Dynamic Light Scattering measurements were done to determine the size of the silver nanoparticles formed is shown in the below figure 8. The particle size distribution peaks determined at various sizes of the particles ranging from 34.50nm to 251.40 nm and had an average particle size of 125.9 nm of synthesized EH-AgNPs as shown in below Figure 8.

Antibacterial Activities

Figure 9 shows the antibacterial activity of Silver nanoparticles By using disc diffusion method, the results of the antibacterial activity of different samples were tested against pathogens are shown in Table.4. The inhibitory activity against positive strains Staphylococcus epidermidis (1mm) and Bacillus subtilis (2 mm) were shown by sample D whereas at sample D, exhibited the antibacterial activity in all the four bacteria. The leaf extract and synthesized nanoparticles manifested better inhibitory actions against pathogens.

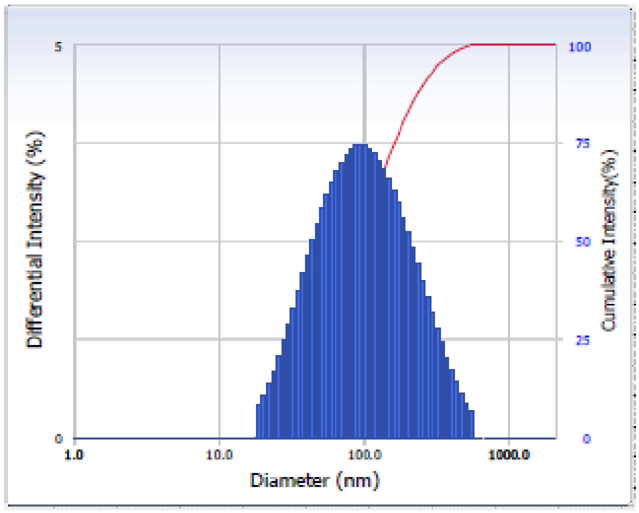
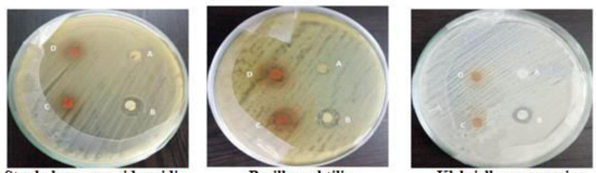


Figure.8 DLS analyses of EH-AgNPs

Table 4: Antibacterial activity of Euphorbia Heteropylla silver nanoparticles

Samples	Concentrations (µl/ml)	Organisms/Zone of inhibition (mm)		
		Staphylococcus epidermidis	Bacillus subtilis	Klebsiella pneumoniae
A (silver nitrate)	10	0	0	0
B (Amoxicillin)	10	8	8	8
C (Plant extract)	10	0	1	0
D (Nanoparticles)	10	1	2	0



Staphylococcus epidermidis Bacillus subtilis Klebsiella pneumoniae

Figure 9 Antibacterial Activities

V. CONCLUSION

Present green synthesis environmentally benign and leaf extract of Euphorbia heteropylla can be used as an effective capping as well as reducing agent for the synthesis of silver nanoparticles. This green physical approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be economic viability, etc. applications of such eco-friendly nanoparticles used to skin problems, fungal disease, abscesses, gonorrhea and body pain. The silver nanoparticles synthesized by Hygrophila Auriculata seed extract were characterized by UV-Visible spectra has confirmed the reduction of silver ions 437 nm. The presence of functional groups was confirmed by Fourier Transform Infrared Spectroscopy. XRD analysis confirms the crystalline face centered cubic structure. The shape and size of the nanoparticles were strongly produced 100-150 nm the Field emission scanning electron microscopy and Energy-Dispersive X-ray spectroscopy has confirmed the presence of absorption peak at 2.98 KeV. DLS study showed various sizes of the particles ranging from 34.50 nm to 251.40 nm and had an average particle size of 125.9 nm. As synthesized AgNPs showed excellent antibacterial activity against pathogenic Two Gram-positive bacterial strains Staphylococcus epidermidis and Bacillus subtilis (MTCC 2451) and One gram negative bacterial strains Klebsiella pneumonia (MTCC 3384).

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