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Green Synthesis of Zinc Oxide Nanoparticles Using Leaf Extract of *Trianthema Portulacastrum*

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ABSTRACT

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for human benefits. Although chemical and physical methods may successfully produce pure, well-defined nanoparticles, these methods are quite expensive and potentially dangerous to the environment. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner. In the present study, synthesis of Zinc nanoparticle using the leaves extract of Trianthema portulacastrum . The phytochemical constituent are detected in the extract of Trianthema portulacastrum .Synthesized of Zinc nanoparticle from extract of Trianthema portulacastrum. The Synthesized of Zinc nanoparticle confirmed by UV –Visible and FTIR analysis. The particle size and shape are find out using SEM . The antimicrobial activity of Zinc nanoparticle isolated from extract of Trianthema portulacastrum is analyzed. The Phytochemical analysis confirm the presence of Alkaloids, terpenoids ,Tannins, Flavonoids and Phenolic compound .The phytosynthesis of Zinc nanoparticles was demonstrated by visual inspection and by performing some spectral techniques (UV-VIS absorption, FTIR spectroscopy and SEM analysis).FTIR results proved that bioactive compounds responsible for Zinc bioreduction could be proteins and flavonoids presumed to act as reducing and capping agents for the ZnO nanoparticles preventing the agglomeration of the particles and thereby stabilizing the nanoparticles.These environmentally benign ZnNPs were further confirmed by using UV-Visible spectroscopy.

Keywords*: Trianthema portulacastrum (leaf), Zinc nitrate,Zinc nanoparticles,UV, FTIR, etc.*

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INTRODUCTION

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical compositions and controlled dispersity and their potential use for human benefits. Although chemical and physical methods may successfully produce pure, well-defined nanoparticles, these methods are quite expensive and potentially dangerous to the environment. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles in an eco-friendly manner[1].

ZnO nanoparticle has attracted considerable interest due to their extensive applicability in various areas such as electronics, catalysis, chemistry, energy and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In the present study, we describe a Nanoparticles were characterized using UV–visible absorption spectroscopy, FTIR, and SEM. SEM analysis showed the spherical nanoparticles with 19.7- 82 nm in size. Further these biologically synthesized nanoparticles were also exhibiting excellent cytotoxic effect on HEp-2 cell lines cost effective and eco-friendly technique for green synthesis of ZnO nanoparticles from 1mM $AgNO_3$ solution through the extract of Piper nigram leaf as reducing as well as capping agent [2] **.**

Metallic nanoparticles are mostly prepared from Nobel metals such as Gold, ZnO, Platinum and Lead using chemical methods. Among the Nobel metals, ZnO (Ag) is the metal of choice in the field of biological systems, living organisms and Since noble metal nanoparticles are widely applied to areas of human contact is a growing need to develop environmentally friendly processes for nanoparticles synthesis. Biological methods of synthesis have paved way for the "greener synthesis" of nanoparticles and these have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization [3]. This has motivated an upsurge in research on the synthesis routes that allow better control of shape and size for various nanotechnological applications. The use of

environmentally benign materials like plant extract bacteria fungi and enzymes for the synthesis of ZnO nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol [4].

The prospect of exploiting natural resources for metal nanoparticle synthesis has become to be a competent and environmentally benign approach. Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles[5]. ZnO nanoparticles have been reported to be synthesized from various parts of herbal plants viz. bark of Cinnamom, Neem leaves, Tannic acid and various plant leaves[6].

Particles in size range 10-9m are known as nanoparticles or sub-micronparticles. They are also known as quantum dots due to quantum property possess by them .

Metal nanoparticles have received significant attention in recent years owing to their unique properties and practical applications. In recent times, several groups have been reported to achieve success in the synthesis of Au, Ag and Pd nanoparticles obtained from extracts of plant parts, e.g., leaves, lemongrass, neem leaves and others**.** These researchers have not only been able to synthesize nanoparticles but also obtained particles of exotic shapes and morphologies[7].The impressive success in this field has opened up avenues to develop "greener" methods of synthesizing metal nanoparticles with perfect structural properties using mild starting materials. Traditionally, the chemical and physical methods used to synthesize ZnO nanoparticles are expensive and often raise questions of environmental risk because of involving the use of toxic, hazardous chemicals[8].

So, the synthesis and design of nanomaterials through biological routes (called biosynhesis) have attracted great interest. Among the biological systems, the living plants are considerably preferred for biosynthesis of ZnO nanoparticles due to the diversity richness of plant kingdom that provides phytochemicals with strong antioxidant properties. It is well known that plants have been used by humans for a very long time to treat many diseases [9].

In recent years plant mediated biological synthesis of nano particles is gaining importance due to its simplicity and eco-friendliness[10]. Several plants serve as potential biological materials for the synthesis of nanoparticles. Among the various inorganic metal nanoparticles, ZnO nanoparticles have received substantial attention for various reasons ZnO belongs to the class of metal oxides, which is characterized by photo catalytic and photo - oxidising capacity against chemical and biological species. The progress of technology and quality of life of mankind has always been closely knit with the progress in material science and material processing technology[11]. Most techniques applied in material processing are based on breaking up large chunk of a material into desired shapes and sizes, inducing strain, lattice defects and other deformations in the processed material. Recent developments and findings in nanotechnology and the demonstration based on various quantum size effects in nanoscale particles, reveals that most of the novel work and devices of the future will be based on properties of nanomaterials[12].

The green synthesis of ZnO nanoparticles have been achieved using P. edulis plant extracts with Zinc Nitrate in this investigation. ZnO nanoparticles have been synthesized using plants with different precursors. The nanoparticles from bio-sources have been utilized effectively in various applications of pharmaceutical industries and biomedical fields[14-15]. Cost effective and non-toxic approaches have proved the potential benefit of biogenesis of nanoparticles, which attracted large scale research in this field. The whole plant mediated synthesis of ZnO nanoparticles could reveal the better understanding of production of nanoparticles of interest. Biogenesis of Zinc oxide nanoparticles using whole plant parts have also been achieved in *Passiflora foetida , Morinda pubescens, Lawsonia inermis* and *Duranta erecta*[16].

MATERIAL AND METHODS

Homogenate was prepared by weighing 20grams of fresh leaves of Trianthema portulacastrum collected from Pattukkoti. Washed thoroughly (thrice) in distilled water and homogenized using a mortar and pestle. The homogenate was then filtered using a sterile gauze cloth. This homogenate extract prepared was then transferred to a sterile container and used for the study.

Preparation of ZnO Solution

The ZnO NPs were prepared according to the earlier report with slight modification 0.02M aqueous Zinc acetate dihydrate was dissolved in 50 mL of deionized water to prepare 0.2 M. The leaf extracts were separated into three sets as 0.25,0.5mL&1mL . In another vessel 2M of NaOH was dissolved in 25 mL of deionized water to get 2M NaOH. In a drop wise manner, NaOH was added to the contents of Zinc acetate dihydrate with continuous stirring, and was subjected to heating at 60degree. In an instant, white precipitate was obtained. The precipitate was then centrifuged at 1000 ppm for 10 min, repeatedly

washed 4 to 5 times with deionized water and then dried at 80°C for 2 hours. The fine powder of ZnONPs obtained was used for the characterization.

Optimization of Various Parameters for Nanoparticles Synthesis

Uv-Visible Spectra Analysis

The bioreduction of ZnO in solutions was monitored by measuring the UV-VIS spectrum of the reaction medium. The UV-VISIBLE spectral analysis of the sample was done by using U-3200 Hitachi spectrophotometer at room temperature operated at a resolution of 1 nm between 200 and800 nm ranges.

FTIR analysis

For FTIR measurements, the ZnO nanoparticles solution was centrifuged at 10,000 rpm for 30 min. The pellet was washed three times with 20 ml of de-ionized water to get rid of the free proteins/ enzymes that are not capping the ZnO nanoparticles. The samples were dried and grinded with KBr pellets and analyzed on a Shimadzu IR-IR Affinity1 model in the diffuse reflectance mode operating at a resolution of 4 cm−1.

Fourier-Transform Spectrometers

Sample Preparation

Instructions vary depending on the type of sample. Liquid samples make good contact with the germanium crystal and do not require any special treatment. Solid samples, on the other hand, do not make good contact. A Pressure Tower on the ATR accessory is used to squeeze solid samples against the crystal surface.

Liquid samples: Apply 1-2 drops of liquid to the center of the germanium crystal with a disposable pipet (avoid contact between the pipet and crystal). Allow the liquid to spread out to make a thin film (it is ok if the liquid spreads across the entire crystal). Leave the Pressure Tower tilted back.

Solid samples: Place solids on the center of the germanium crystal with a micro spatula (avoid contact between the spatula and crystal). Carefully bring the Pressure Tower upright by pulling out the Zno release knob and tilting the Tower forward (do not let the tip fall on the crystal).

Use your spatula to position your sample underneath the Tower's pressure tip. Rotate the knob on top of the Tower clockwise so that the pressure tip presses your sample onto the germanium crystal. Stop rotating the knob when you hear a “click”. These “clicks” are created by a slip-clutch safety mechanism that prevents the tip from applying too much pressure to the crystal.

Typical operation involves the following steps

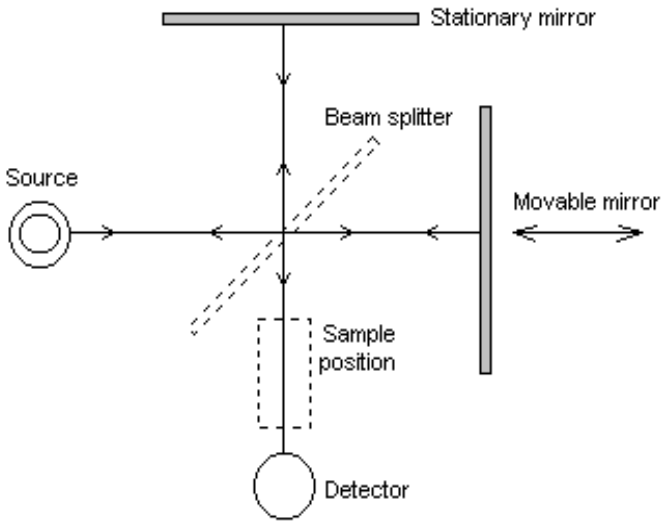
- Setup spectrometer configuration
- Collect background spectrum
- Apply sample to ATR accessory
- Collect sample spectrum
- Find major peaks
- Print spectrum
- Clear sample spectrum and report
- Clean ATR accessory

Any waveform can be shown in one of two ways either in frequency domain or time domain. Dispersive IR instruments operate in the frequency domain. There are, however, advantages to be gained from measurement in the time domain followed by computer transformation into the frequency domain.

If we wished to record a trace in the time domain, it could be possible to do so by allowing radiation to fall on a detector and recording its response over time. In practice, no detector can respond quickly enough (the radiation has a frequency greater than 10^{14}Hz). This problem can be solved by using interference to modulate the IR signal at a detectable frequency. The Michelson interferometer is used to produce a new signal of a much lower frequency which contains the same information as the original IR signal. The output from the interferometer is an interferogram.

The Michelson interferometer:

Radiation leaves the source and is split. Half is reflected to a stationary mirror and then back to the splitter. This radiation has travelled a fixed distance. The other half of the radiation from the source passes through the splitter and is reflected back by a movable mirror. Therefore, the path length of this beam is variable. The two reflected beams recombine at the splitter, and they interfere (e.g. for any one wavelength, interference will be constructive if the difference in path lengths is an exact multiple of the wavelength. If the difference in path lengths is half the wavelength then destructive interference will result). If the movable mirror moves away from the beam splitter at a constant speed, radiation reaching the detector goes through a steady sequence of maxima and minima as the interference alternates between constructive and destructive phases.



If monochromatic IR radiation of frequency, f (IR) enters the interferometer, then the output frequency, f_m can be found by,

$$f_m = \frac{v}{1.5 \times 10^{11}} \times f(ir)$$

where v is the speed of mirror travel in mm/s

Because all wavelengths emitted by the source are present, the interferogram is extremely complicated. The moving mirror must travel smoothly; a frictionless bearing is used with electromagnetic drive. The position of the mirror is measured by a laser shining on a corner of the mirror. A simple sine wave interference pattern is produced. Each peak indicates mirror travel of one half the wavelength of the laser. The accuracy of this measurement system means that the IR frequency scale is accurate and precise.

In the FT-IR instrument, the sample is placed between the output of the interferometer and the detector. The sample absorbs radiation of particular wavelengths. Therefore**,** the interferogram contains the spectrum of the source minus the spectrum of the sample. An interferogram of a reference (sample cell and solvent) is needed to obtain the spectrum of the sample.

After an interferogram has been collected, a computer performs a Fast Fourier Transform, which results in a frequency domain trace (i.e. intensity vs. wavenumber) that we all know and love.

The detector used in an FT-IR instrument must respond quickly because intensity changes are rapid (the moving mirror moves quickly). Pyroelectric detectors or liquid nitrogen cooled photon detectors must be used. Thermal detectors are too slow. To achieve a good signal to noise ratio, many interferograms are obtained and then averaged. This can be done in less time than it would take a dispersive instrument to record one second.

Scanning Electron Microscopy

The supernatant from the maximum time-point of production of ZnO nanoparticles0 was air-dried. The synthesized ZnO nanoparticles were fabricated on a glass substrate were done for the determination of the formation of ZnO nanoparticles. The morphology and size of ZnO nanoparticles was investigated using Scanning Electron Microscope (VEGA 3 TESCAN).The micrograph were recorded by focusing on clusters of particles

RESULT AND DISCUSSION

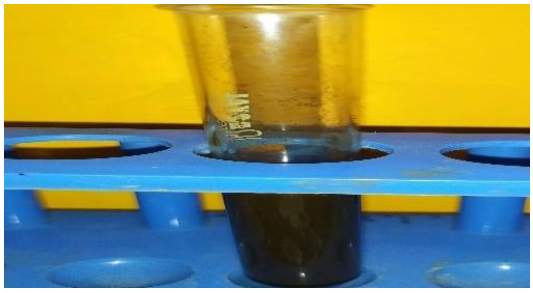


Fig. 1 Synthesised Zno nano Particle (ZNP)

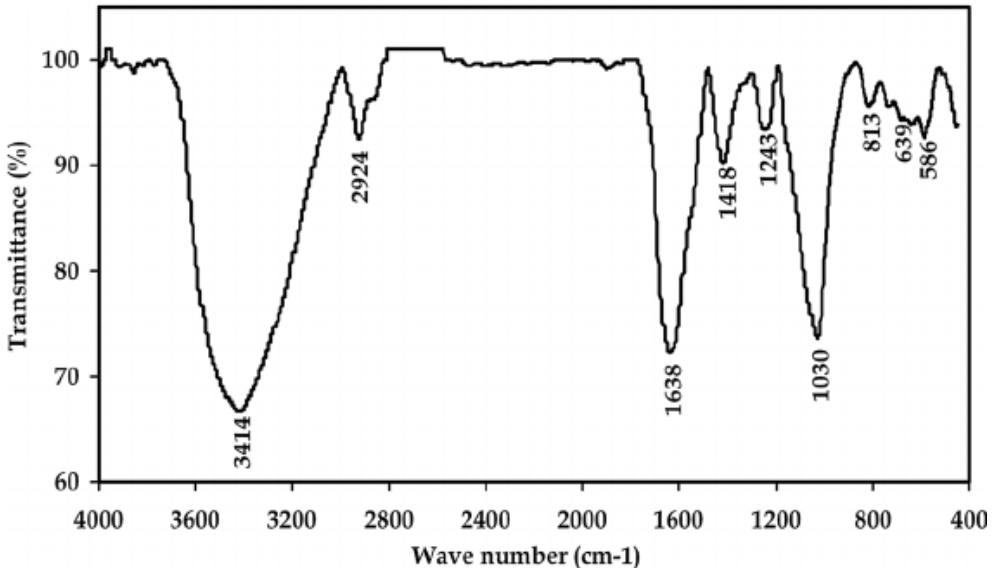


Fig. 2 FTIR RESULT FOR EXTRACT OF TRIANTHEMA PORTULACASTRUM

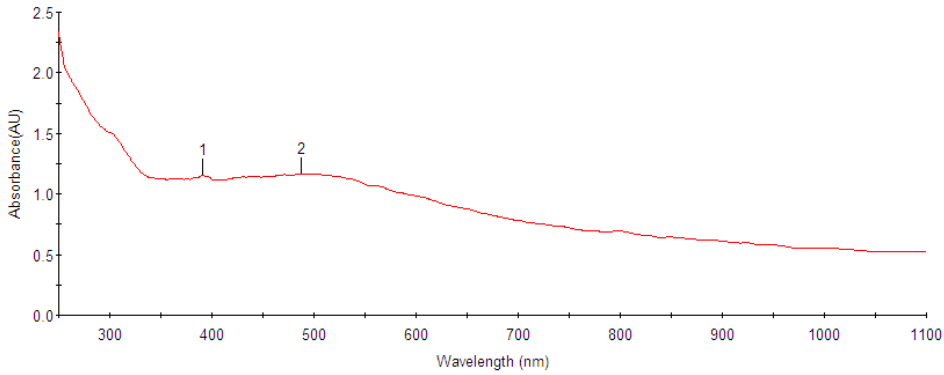


Fig. 3 UV- Vis- Spectroscopy

Detection and Characterization of Phyto ZnO Nanoparticles

Visual Observation: After treatment of Trianthema portulacastrum extract with oxide, the colour change of the reaction mixture was visually observed. The time taken for the reaction mixture to change colour was noted.

The reduction of ZnO ions into ZnO particles during exposure to the plant extract is followed by colour change from colorless or pale yellow to yellowish brown. It is well known that ZnO nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in ZnO nano particles .

UV- Visible- Spectroscopy

Electromagnetic radiation such as visible light is commonly treated as a wave phenomenon, characterized by a wavelength or frequency. Wavelength is defined on the left below, as the distance between adjacent peaks (or troughs), and may be designated in meters, centimeters or nanometers (10-9 meters). Visible wavelengths cover a range from approximately 400 to 800 nm. Optical properties of the as-prepared ZnO nanostructure sample was revealed by UV Visible spectrum and photoluminescence spectroscopy at room temperature, as shown in Figure No.1. It can be seen from the Figure No.1 that there was intensive absorption in the ultraviolet band of about 300-1100 nm. UV-Visible spectroscopy is usually conducted to confirm the synthesis of ZnO NPs. Electrons start oscillating at a certain wavelength range due to surface plasmon resonance (SPR) effect.

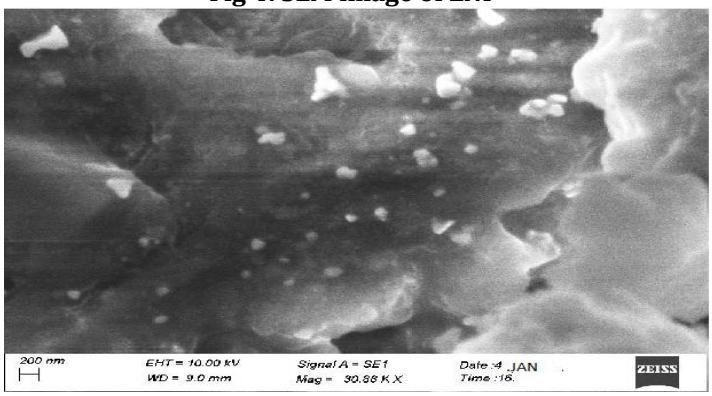
Represents the UV-Visible spectra of freshly prepared ZnO NPs. Peak obtained at 380 nm clearly demonstrates the pres- ence of ZnONPs in the reaction mixture. Initial peak obtained at range of 420 nm got further raised due to oscillation of more electrons after 5 h which depicts the continuous synthesis of ZnONPs.

FTIR analysis:

Two milligram of ZnO nanoparticales were prepared by mixing with 200 mg of spectroscopic grade KBr. FTIR spectra were recorded using a Nicolet 520P spectrometer with detector at 4000-400 cm-1resolution and 20 scans per sample. FTIR Spectra of aqueous Zinc Oxide nanoparticles prepared from the seaweed extract was carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by plant extract .

The results of the FTIR spectrum of hot and cold methods of seaweed extracts of Zinc Oxide nanoparticles are depicted in Figure 1and 2. The band at 437-445 cm-1 and 509-511cm-1 is attributed to ZnO nanoparticles. The broad peak at 3402-3419cm-1 correspond to O-H band and C=O indicating the compound to be aliphatic carboxylic acid. The band at 1554-1558cm-1 is attributed to the presence of aromatic ring. The band at 1028-1033cm-1 correspond to saturated primary alcohol. The band at 2927-2931cm-1 is due to doublet absorption of C-H stretching vibration of an aromatic aldehyde. These bands are indicative of terpenoid group of compounds present in aqueous *Trianthema portulacastrum* extract [12]. From FTIR analysis, it can be inferred that alcohols, terpenoids ketones, aldehydes and carboxylic acid were surrounded by synthesized nanoparticles. Phenolic compounds flavonoids, lignans, coumarins, tannins, quercetin, alkaloids, cynogenic glycosides present in the leaves formed a strong capping on the nanoparticles [2]. The prominent doublet absorption at 2927. The FT-IR studies clearly indicates the reduction and capping agents ie. biomolecules present in the Trianthema portulacastrum leaf extract.A very ignorable peak obtained at 650.01 and 532.35 demonstrated the probable presence of C-Alkyl chloride and Hexagonal phase ZnO.

Fig 4: SEM image of ZNP



CONCLUSION

Nanotechnology finds extensive applications in nanomedicine, an emerging new field. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical composition and controlled dispersity and their potential use for human benefits. Nanoparticles can be synthesised by chemical and physical methods but these methods are quite expensive and toxic. Use of biological organisms, plant extracts could be an alternative method for production of nanoparticles.

- The Phytochemical analysis confirm the presence of Alkaloids, terpenoids ,Tannins, Flavonoids and Phenolic compound .
- The phytosynthesis of Zinc nanoparticles was demonstrated by visual inspection and by performing some spectral techniques (UV-VIS absorption, FTIR spectroscopy and SEM analysis).
- FTIR results proved that bioactive compounds responsible for Zinc bioreduction could be proteins and flavonoids presumed to act as reducing and capping agents for the ZnO nanoparticles preventing the agglomeration of the particles and thereby stabilizing the nanoparticles.
- These environmentally benign ZnNPs were further confirmed by using UV-Visible spectroscopy.

To conclude we have used unreported, inexpensive, nontoxic, ecofriendly and abundantly available *Trianthema portulacastrum* for the rapid synthesis of ZnO NPs in the range of 9-10nm. FT-IR studies of aqueous *Trianthema portulacastrum* extract reveals the presence of phyto constituents like alcohol, aldehyde and amine which were the surface active molecules stabilized the nanoparticles and this phytochemicals have interacted with the Zinc surface and aids in the stabilization of Zinc Oxide nanoparticles. This green synthesis approach shows that the environmentally benign and renewable *Trianthema portulacastrum* extract can be used as an effective stabilizing as well as reducing agent for the synthesis of Zinc Oxide nanoparticles.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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