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**ORIGINAL ARTICLE**

# Nanophytosynthesis and Characterization of Silver Nano Particles Using *Chrysanthemum parthenium* Extract as an Eco-Friendly Method

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**KEYWORDS**

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**ABSTRACT:** The current study (in Khalkhal, 2014) deals with a simple nano phytosynthesis approach to produce silver nanoparticles (AgNPs) using a methanolic extract of the leaves of *Chrysanthemum parthenium* along with evaluation of the corresponding physicochemical properties. Accordingly, AgNPs were analyzed and characterized by UV/Vis spectrophotometry and FT-IR spectroscopy. In addition, the morphology and size of the reduced silver nanoparticles were determined by the transmission electron microscope (TEM), scanning electron microscope (SEM) and X-ray diffractometer (XRD) instrumentations. The FT-IR spectra of plant methanolic extract taken before and after synthesis of nanoparticles, showed the possible functional groups for the formation of AgNPs. Moreover, the SEM accounted for the formation of AgNPs with a mean size of 43.1 nm. The TEM study also confirmed the formation of AgNPs over the average size of 30-50 nm. Finally, the XRD pattern showed that the silver nanoparticles are crystalline in nature with a face-centered cubic (FCC) structure and a mean size of 46.7 nm. The suggested approach involving nanophytosynthesis of AgNPs using the leaves of *C. parthenium* serves as a beneficial, effective, rapid, eco-friendly and easy alternative to conventional synthetic methods.

## INTRODUCTION

Nowadays, nanotechnology is a growing field of science more specifically due to its usage in different disciplines of technology and for fabrication of new materials at the nanosize level [1]. Nanophytosynthesis of nanoparticles in an eco-friendly manner is a quest for current science, because there is a ubiquitous need for these particles. Synthesis and characterization of nanomaterials is presently an important subject of research, as selection of size and shape of nanoparticles provides an efficient control over many of the chemical and physical properties [2].

The Ag nanoparticles with various and important applications have gained much attention for their incomparable nature and advantageous use on various examinations [3, 4].

One of the most important branches of biosynthesis of nanoparticles involves the utilization of extracts from diverse plant materials to the biosynthesis reaction, such as *Mucuna pruriens* [5], *Cas-*

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*sia fistula* [6], *Cassia occidentalis* [7], *Desmodium triflorum* [8], *Cinnamomum zeylanicum* [9], *Gliricidia sepium* [10], *Opuntia ficus- indica* [11], *Clerodendrum inerme* [12], Banana peel [13], *Magnolia kobus* [14], *Azadirachta indica* [15], etc. According to the literature, the *Camellia sinensis* extract (green tea) has been used to reduce and stabilize of produced Ag nanoparticles and Au nanostructures in an aqueous solution at ambient conditions [16]. Recently, medicinal plants [17], fruits [18], weeds [19] and spices [20] have been used for production of nanoparticles. Green synthesis of silver nanoparticle using *Acanthus ilicifolius* extract has been reported and the respective biological activities have been evaluated against *Armigeres subalbatus* and *Aedes aegypti* mosquito larvae [21].

The biosynthesis of silver nanoparticles by reducing an aqueous solution of $AgNO_3$ using a hydro-alcoholic extract from the leaves of *Viburnum lantana* and their biological activities were reported from Iran [22]. The root extract of *Justicia adhatoda* played an important role both as a reducing and a stabilizing agent in the synthesis of silver nanoparticles, as well [23].

The most widely used and known applications of silver and Ag-NPs are found mainly in the medical industries. These include topical ointments and creams containing silver to prevent infection of burns and open wounds [24]. It has been also reported that low levels of AgNPs are fatal for microorganisms. However, there is no risk for the cells of human body in these small concentrations [25].

The genus *Chrysanthemum* (syn. *Tanacetum*) (Family: Compositae) is represented in Iran by twenty-six species of which 12 species are endemic to the country [26]. Feverfew, the common name of *C. parthenium* (L) Schultz Bip, is a traditional herb used in Iran and Europe as a powerful sedative remedy against the symptoms of migraine, arthritis and psoriasis. It can also inhibit blood platelet congeries [27-29]. In addition, this medicinal plant serves as an insect repellant with prominent antibacterial, antioxidant and antifungal activities [30, 31].

To the best of our knowledge, there is no report in the literature concerning the synthesis of silver nanoparticles by using feverfew (leaves) extract. In the present study, we have successfully synthesized and investigated silver nanoparticles from the *C. parthenium* leaf extract and ascertained their characterization. We used the methanolic extract from the leaves of this plant since *C. parthenium* has a widespread distribution in many districts of Iran, specifically in Khalkhal area, Ardabil Province, Iran.

## MATERIALS AND METHODS

### *Materials and Preparation of Plant Extract*

The leaves of *C. parthenium* were collected on 29 June 2013 in Khalkhal-Asalem road Northwestern of Iran, at an altitude of 2150 m and dried for 5 days at room temperature. A voucher sample was kept at the Herbarium of Agriculture Research in Ardabil Center (HARAC), Iran. Methanol ($CH_3OH$, 95.9%), silver nitrate ($AgNO_3$, 99.9%), double distilled water were prepared from Mojallali Company (Iran). All reagents used were of analytical reagent grade. Dried and finely powdered *C. parthenium* leaves (100g) were totally moistened with methanol to proceed 12 g of raw extract after vaporization of the alcohol in reduced pressure. The condensate extract was then kept in black vessel at 4 °C until utilization.

### *Phyto-assisted Synthesis of Silver Nanoparticle*

For preparation of silver nano scales, 5g-portions of raw extract of plant leaves (*C. parthenium*) were added to double distilled water (100 CC) under a vigorous shaking for about 1 h. This step was followed by addition of 100 mL of a 0.5 M solution of silver nitrate ($AgNO_3$). The resulting solution was blended at laboratory temperature (25 °C) for 48 h. The silver nitrate was used as a pre-

cursor in the synthesis of Ag nanoparticle. AgNPs were afforded during the reaction time. During the stirring step in the phytosynthesis procedure, an observable change in the mixture color was noted that was stable for about 5 to 10 minutes. The color changes represented the production of silver nanoparticles. The AgNPs admixture obtained was subsequently purified and laundered by repeated centrifugation at 2000 rpm for 0.5 h.

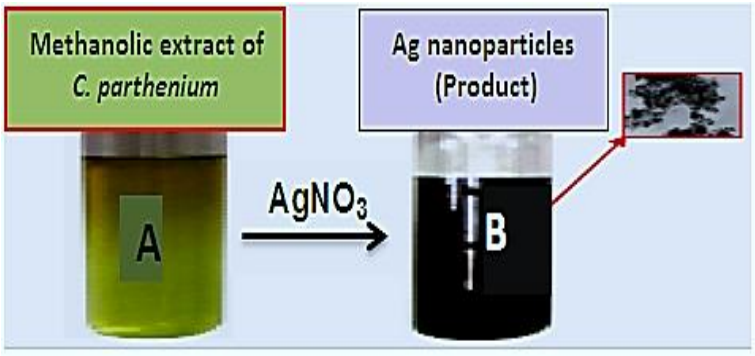


**Figure 1.** Color changing before reaction (**A**) and after reaction (**B**) in the nanophytosynthesis process of Ag NPs.

***UV-VIS Spectra Analysis***

The bioreduction of pure silver ions ($Ag^+$) in solutions to Ag nanoparticles were monitored by measurement of the UV-Visible spectral data of the reduction medium at different time intervals taking 1 mL of the samples, versus 1 mL of deionized water as blank. UV/Vis data resolution has been accomplished by using a Perkin Elmer-Lambda 25 UV/Vis spectrophotometer operated at 1 nm intervals ranging from 200 to 800 nm. In this relation, the absorption signals in a wavelength range of 420-450 nm were considered, which are identical to the characteristic UV/Vis spectrum of metallic Ag representing the successful synthesis of silver nanoparticles.

***SEM and EDX Analysis of Silver Nanoparticles***

After successful synthesis of the AgNPs, the SEM analysis was accomplished using the LEO (1430VP, SEM apparatus) capable of operating at a precipitation voltage of 10 keV. 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. Afterwards, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under the irradiation of a mercury (Hg) lamp for 5 min. For EDX resolution, the presence of silver metal in the sample was dried and drop coated was placed onto carbon film. EDX analysis was then effected using the instrument thermal energy dispersive X-ray analysis (EDX) combined with SEM.

***TEM Resolution of AgNPs***

Transmission electron microscopy (TEM) imaging was carried out using Philips GM-30 electron microscope. In a preliminary step, sample was interspersed in double distilled water. Then, a drop of thin dispersion was sited on a staining mat. In the next step, carbon-coated copper grid was enclosed into the drop with the coated side upwards. The grid is picking upped and air dried after about 10 min. The silver nanoparticles size dispensations were specified using the UTHSCSA Image Tool ver. 3.00 programmed.

***XRD Analysis***

The XRD analysis of drop-coated films of Ag nanoparticles in sample was accomplished for the determination of the formation of Ag nanoparticles by a Bruker, B8-advance, X-ray diffractometer operating at a voltage of 40 kv and a current of 30 mA with Cu Kα radiation. The diffracted intensions were finally recorded 10 º -90 º at 2θ angles [32].

***FT-IR Measurements***

To recognize the possible biomolecules responsible for the reduction of the silver cations ($Ag^+$) and to cover the bio-reduced AgNPs generated by the herbal material hydromethanolic extract, some complimentary evaluations were carried out based upon the FT-IR spectroscopy. AgNPs powder piece was obtained by centrifuging the synthesized Ag nanoparticles mixture at 2000 rpm for 40 min. The pellet including AgNPs was dispersed with sterile double distilled water 3 times to eliminate the unbound biological contaminations and obviate the free amino acid, proteins/enzymes that are not covering ligand for the AgNPs. The obtained nano silver particles were finally collected and dried in an oven at 60 °C and ground with KBr pellets and analyzed on a Bruker Tensor 27 model in the spread replication mode operating at a resolution of 4 $cm^{-1}$.

## RESULTS AND DISCUSSION

***Silver Reduction***

The biosynthesis of Ag nanoparticles (AgNPs) via an organic extract of plant leaves (*C. parthenium*) were carried out. Ag nanoparticles were synthesized from a silver nitrate solution containing silver ions ($Ag^+$) by treating them with the corresponding seeds extract. In our experiments, silver nitrate ($AgNO_3$) was used as a reducing agent since silver has recognizable properties such as good catalytic, chemical stability and well conductivity. The aqueous $Ag^+$ cations when subjected to plant materials were reduced in admixture, thereby transduction to the establishment of silver hydrosol occurred in the reaction medium. By reduction of silver cations ($Ag^+$), the formation of silver nanoparticles was started and continued within the reaction. The pictures of extract from *C. parthenium* leaves (photograph A) before and after the phytosynthesis reaction with $AgNO_3$ and color changes during the conversion of $Ag^+$ cations to Ag nanoparticles (photograph B) have been represented in the Figure 1. The emersion of a tawny brown color confirms the entity of silver nanoparticles in the flask (B). Also, the Ultraviolet/Visible spectrophotometry could be used to test the size and shape controlled nanoparticles in aqueous suspensions [33].

***UV/VIS and FT-IR Spectra Analysis***

The UV/Vis spectroscopy is an efficient method to verify the formation and stability of AgNPs. The synthesis of AgNPs had been confirmed by considering two criteria including i) color changes and ii) UV/Vis spectrum of the reaction media (Figure 2-A).

The UV/Vis spectrum of colloidal mixture of AgNPs obtained from *C. parthenium* leaves extract have absorption peaks at 350 to 460 nm and the observed peak broadening indicated that the nanoparticles were polydispersed.

Recently, the presence of biflavonoid and methoxy groups in the extract of *C. parthenium* as a major compound play a key role in the reduction and stability of AgNPs [34]. These kinds of flavonoid derivatives are known to act as potential reducing agents by donating of free electrons [35]. A remarkable enhancement in the production of AgNPs was seen with an increase in the reaction time. The concentration of the *C. parthenium* leaves extract also act an important role as it is

responsible for the synthesis of symmetrical AgNPs.

The nanoparticle compounds can be prepared by reducing metal cations through mediating some chemical compounds in biosynthesis, the plant materials including flavonoid derivatives, flavonol glycosides, coumarins, xanthones, triterpenes, glycosides, lactones, anthraquinones, phloroglucinols, pyrones, lipids, tannins and essential oils acts as reducing acting for the synthesis and efficiency of nanoparticles [36-38].

The recent findings of *Capsicum annuum* L. extract [39], *Diopyros kaki*, *Pinus desiflora*, *Magnolia kobus*, *Ginko biloba*, and *Platanus orientalis* extract [40] characterized that the proteins and amino acids which have amine groups (NH and $NH_2$) played a reducing role during the preparation of Ag nanoparticles in the mixture, and that the secondary structure of the proteins exchanged after reaction with silver cations ($Ag^+$).

FT-IR spectrum of the crude extracts obtained from a hydroethanolic extract of *C. parthenium* (leave) derived before and after preparation of Ag nanomaterials were analyzed which communicated for the possible action groups in their production.

The FT-IR spectrum of synthesized silver nanoparticles by using *C. parthenium* leaf extract is shown in Figure 2-B. This figure shows the FT-IR spectrum of this herbal extract which represents absorption signals at 3415, 2925, 1660, 1385, and 1045 $cm^{-1}$.

The scapular signal at 1660$cm^{-1}$ is assigned to the carbonyl group (C=O). The signal at 3415 corresponds to O-H, N-H and C-H stretching vibrations of alcohols, amides and alkanes, respectively or H-bonded to phenolic compounds. To identify the biochemical components for reduction and efficient stabilization of the metal nano structures, the signal at 3415 $cm^{-1}$ corresponds to O-H, as also the H-bonded alcoholic and phenolic compounds. The band occurring at 2925 is related to C-H stretching vibration. The peak at 1660 $cm^{-1}$ accounts for the stretching vibration of C=O on chromen-4-one rings of flavonoid derivatives. The signals at 1385 belong to C-H in-plane bend to alkenes, as well.

An immediate reduction of silver ions to silver nanoparticles in the present study might have resulted due to water-soluble phytochemicals like flavonoids, quinones, and organic acids present in the *C. parthenium* leaf.

Ag reduction and fabrication of its nanoparticles accomplished due to phytochemical compounds present in this species may be considered as the highlight and novelty of this investigation.

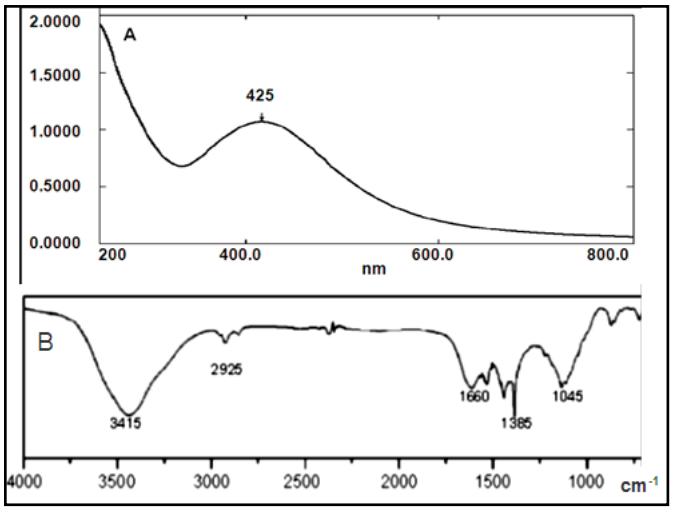


**Figure 2.** (A): Absorption peak of Ag-NPs extract of *C. parthenium* leave under UV/Vis spectroscopy.
(B): FT-IR spectrum of *C. parthenium* leave extract.

***SEM, EDX and TEM Studies***

The morphology of the silver nanoparticles was determined by SEM. The SEM picture characterized relatively cubic and almost orbicular shape nanoparticle figured with size 43.1 nm (Fig. 3-A). The scanning electron microscopy (SEM) determinations of the piece showed the preparation of AgNPs, which were confirmed to be of silver by Energy Dispersive Analysis of X-rays (EDX) spectrum denoting the presence of silver metal in synthesized nanoparticles.

As shown in Figure 3(A and C), good-dispersed NPs could be observed in the samples treated with $AgNO_3$. Analysis of the EDX also showed a signal in the silver area to affirm the formation of AgNPs (Figure 3-C). The optical absorption signal is seen approximately at 3 keV, which is a typical for the absorbance of metallic AgNPs due to surface plasmon resonance (SPR) [41].

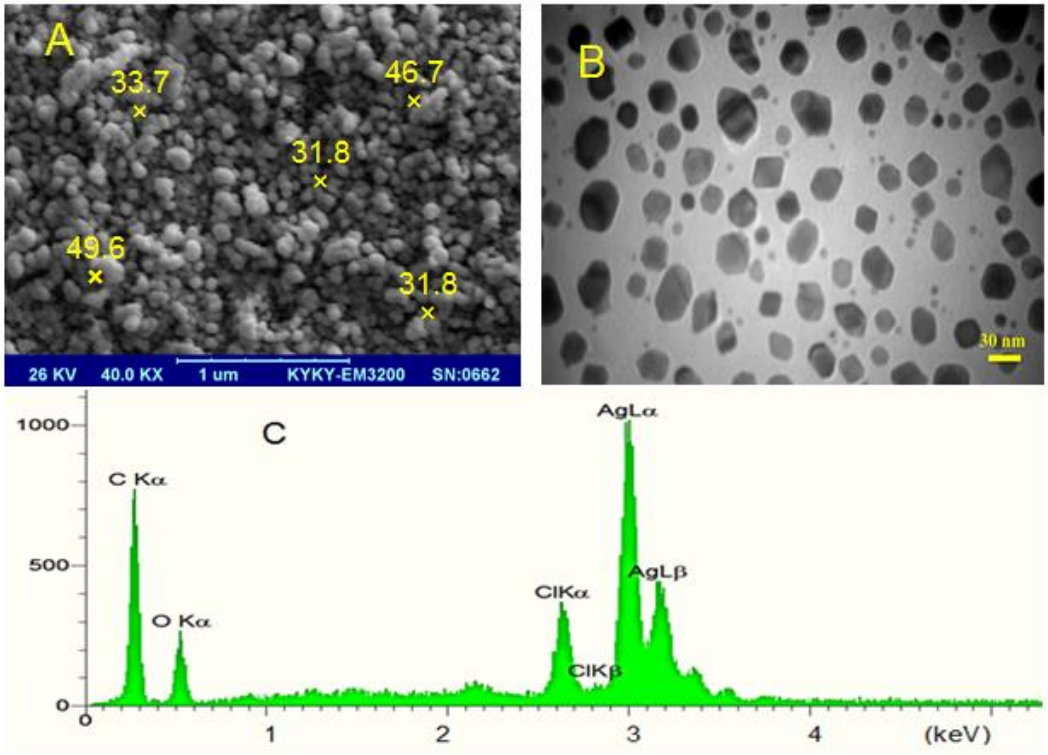


**Figure 3.** (A) SEM, (B) TEM and (C) EDX analysis of AgNPs synthesized by *C. parthenium* leave.

The size, morphology and crystalline nature of the synthesized AgNPs were investigated and determined by the transmission electron microscopy (TEM) images. The typical TEM image of as biosynthesized silver nanoparticles is shown in Figure 3-B, which indicates that the AgNPs developed were well formed. This photo and size dispensations of AgNPs characterized that the mean size of the synthesized materials were approximately over the ranged 30 to 50 nm. They were spherical and cubic like in shape and few nanoparticles were agglomerated (Figure 3-B).

***XRD Analysis***

The X-ray diffraction (XRD) measurement was used to confirm the crystalline nature and size of the synthesized silver nanoparticles. The dry powders of the prepared nano scale particles were used for XRD analysis. Figure 4 shows a representative XRD pattern of the AgNPs prepared by *C. parthenium* leave extract after the complete reduction of $Ag^{+}$ cations to $Ag^{o}$.

The diffraction pattern was recorded by Cu-K$\alpha_1$ radiation with a $\lambda$ of 1.5418 A° in the region of $2\theta$ from 10º to 90º at 0.02º/ min considering a time constant of 2 s. A number of strong diffracted peaks observed at 77.45º, 64.53º, 81.54º, 38.17º and 44.33º and can be ascribed to the (311), (220), (222), (111) and (200) crystalline planes of the FCC crystalline structure of AgNPs were obtained, respectively [42].

The size of the nanoparticles was calculated through the Debye-Scherrer's equation. The crystalline nature of silver nanoparticles was studied with the aid of X-ray diffraction as shown in Figure 4. The average diameter of particles of the silver nanoparticles was found to be 46.7 nm obtained from the FWHM of signal ascribed to 111 planes and recognized by using the following equation (Debye-Scherrer's equation):

$$D = K.\lambda / \beta.\ Cos\theta \qquad \text{(eqn.1)}$$

Where D and $\lambda$ are respectively the average grain size of crystallite and the X-ray wavelength (1.5418Å), while $\beta$ implies the diffracted full width of the XRD peak at half maximum (FWHM in radians).

The mean diameter of the synthesized nanoparticles determined by XRD line broadening was in good agreement with those obtained from SEM. From XRD and SEM, the average particle sizes were found to be nearly 46.7 and 43.1 nm, respectively.

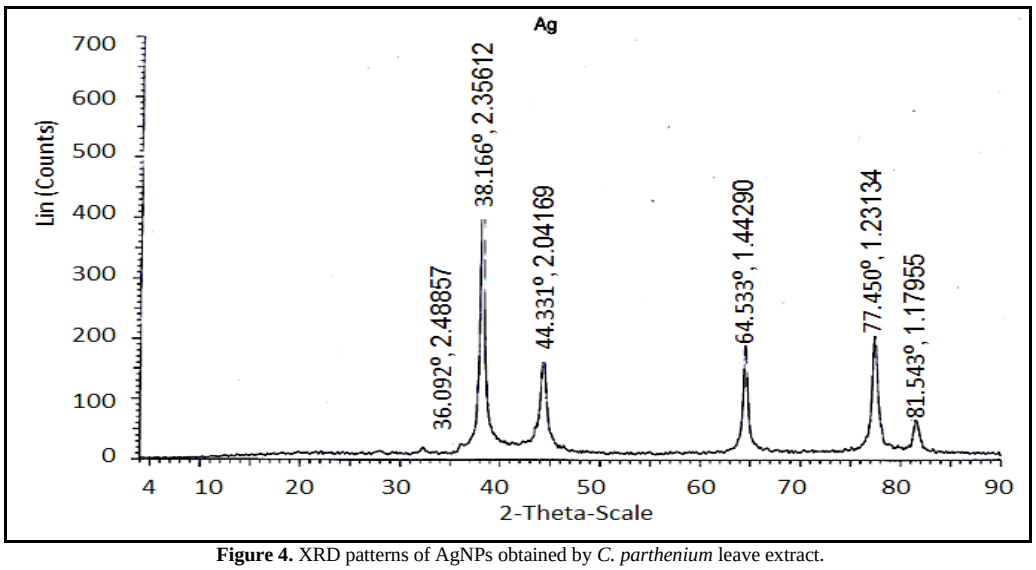


**Figure 4.** XRD patterns of AgNPs obtained by *C. parthenium* leave extract.

## CONCLUSIONS

In the present report, the silver nanoparticles have been synthesized by a hydromethanolic extract from the leaves of *C. parthenium*. The proposed methodology was found to be an efficient, economical, rapid and eco-friendly process. This study included the biosynthesis of AgNPs from silver ions ($Ag^{+}$) by using *C. parthenium* extract. Our systematic analysis on the nanoparticles structure, morphology and size by XRD, TEM and SEM together with the chemical composition by

FT-IR and UV/Vis, strongly suggests the formation of silver nanoparticles. From the TEM analysis, the average sizes of the nanoparticles were found to be 30-50 nm. FT-IR and SEM measurements provided evidence for the existence of flavonoids and protein to form a coat covering on the silver nanoparticles to stabilize and prevent the agglomeration of the silver particles. The potential use of such secure and eco-friendly nano materials in wound healing, bactericidal and other medical, drug, dress and electronic usage, makes this method as an alternative approach for the large-scale synthesis of other nanomaterials. As a concluding remark, it seems that the nano phytosynthesis method for metal nanoparticles such as AgNPs is a convenient, suitable, rapid, low cost, easily scale up and eco-friendly process.

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