



## GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *MARSILEA MINUTA LINN* AND EVALUATION OF ITS ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES

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### ABSTRACT

Nanoparticles of silver nitrate are receiving increasing attention due to their widespread applications. The present study deals with the biosynthesis and characterization of silver nanoparticles (AgNPs) using aqueous leaf extract of *Marsilea minuta Linn* and its antioxidant and antibacterial activities. Parameters like concentrations of reactants, silver nitrate and whole plant extract were standardized to produce silver nanoparticles at 37°C. The maximum synthesis of AgNP was obtained in 5% plant extract, pH 7 and 1 and 3 mM silver nitrate within 30 minutes at 37°C. AgNPs were characterized by using UV-visible Spectroscopy, Scanning electron microscopy and Fourier Transform Infrared spectroscopy. Silver Surface Plasmon Resonance (SPR) occurred at 437 nm for 1 and 3 mM AgNP's. SEM analysis showed the presence of nanoparticles in spherical shape and dispersed and the size ranged from 53-95 nm. The FTIR spectra revealed the presence of reducing groups in the extract responsible for AgNP synthesis. The synthesized AgNPs exhibited super oxide, hydroxyl radical scavenging and ferrous chelating activities. The AgNPs exhibited antibacterial activity against pathogenic bacteria *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Salmonella typhi* and *Bacillus subtilis* which was analysed by Disc diffusion method. Antioxidant and antibacterial activity of AgNP's of *Marsilea minuta* showed maximum activity in 3mM AgNP. Ferrous chelating and hydroxyl radical activity was observed in AgNP's (3mM) of *M. minuta* with an IC<sub>50</sub> value of 100 & 500µg respectively. The highest zone of inhibition was observed in 3mM AgNP's of *M. minuta* against *Klebsiella pneumoniae* and *Enterococcus faecalis* with an inhibition zone of 13.3 and 13.0 mm respectively.

**KEYWORDS:** *Marsilea minuta*; Silver nanoparticle; Antioxidant activity; SEM; FTIR; Antimicrobial activity



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## INTRODUCTION

Nanotechnology is one of the most active areas of research in modern material science and technology. Nano particles possess unique electrical, optical as well as biological properties and are applied in catalysis, biosensing, imaging, drug delivery, nanodevice and fabrication<sup>1</sup>. Plant mediated synthesis has been suggested possibly as an eco-friendly, cost effective and less time consuming process and does not involve any toxic chemicals<sup>2-3</sup>. *Marsilea minuta* L., a common aquatic or sub-aquatic fern well-known as "susnisak" in Tripura, aaraikeerai in Tamilnadu, India, is a member of Marsileaceae family. The plant is widely used in different traditional and folk medicinal systems for its medicinal value, and recommended for the treatment of psychopathy, diarrhea, respiratory diseases, and skin diseases<sup>4-6</sup>. Leaves of *M. minuta* are prescribed by folk medical practitioners to treat diabetes and gastrointestinal disorders. The present work was undertaken to assess the antioxidant and antimicrobial activities of AgNPs synthesized from the leaf extract of *M. minuta* using silver nitrate.

## MATERIALS AND METHODS

The plant *Marsilea minuta* was collected from from the Lachivakkam village, Thiruvallur district, Tamilnadu and was authenticated by Professor P. JAYARAMAN, Ph.D. Director, Institute of Herbal Botany, Plant Anatomy Research Centre, West Tambaram, and Chennai (Reg no: PARC/2017/3435).

### **Preparation of aqueous extract of the *Marsilea minuta***

5 grams of the leaf powder of *Marsilea minuta* L. were weighed and mixed with 100ml of distilled water. Aqueous extract was obtained by the filtration with Whatman number1 filter paper and made up to 100 ml with distilled water. The extracts was stored at 4°C and used within 15 days.

### **Preparation of Silver nitrate**

One millimolar (1mM) silver nitrate (Merck Grade) was prepared by dissolving 0.0421g of AgNO<sub>3</sub> in 250 ml of the distilled water and stored in amber bottle to prevent auto oxidation of silver.

### **Biosynthesis of Silver Nanoparticles**

To 0.5ml of the extract (5%), 9.5ml of 1 and 3mM concentrations of AgNO<sub>3</sub> was added and maintained at 37°C for 1hr. The color change of the solution was observed which indicate the preliminary confirmation for the formation AgNP.<sup>7</sup>

### **Characterization of Silver Nano particles UV-Visible Spectroscopy**

UV-visible Spectroscopy was recorded using Thermo Scientific Evolution 201 UV-vis Spectrophotometer. A small aliquot was drawn from the reaction mixture and a spectrum was taken on a wavelength from 200 - 800 nm.

### **Fourier Transform Infra red Spectroscopy (FTIR)**

FTIR was carried out to identify the possible biomolecules responsible for the reduction of silver ions using Bruker-Alpha spectrometer.

### **Scanning Electron Microscopy (SEM)**

The size and shape of the biosynthesized nanoparticles were observed by SEM.

### **Biological Activities of the AgNPs of *Marsilea minuta***

#### **Antioxidant scavenging activities**

The effect of nano particle on hydroxyl radical was estimated using the method (Halliwell *et al.*)<sup>8</sup>. Superoxide scavenging activity was determined by alkaline dimethyl sulphoxide method<sup>9</sup> Ferrous chelating activity was measured by the method (Huang *et al*)<sup>10</sup>.

#### **Antibacterial activities**

Antibacterial activity of biologically synthesized AgNPs of *M. minuta* was determined by Disc diffusion method on nutrient agar medium.<sup>11</sup> Pathogenic bacteria *Staphylococcus aureus*, *Klebseilla pneumoniae*, *Enterococcus faecalis*, *Salmonella typhi* and *Bacillus subtilis* obtained from PG and Research Department of microbiology, DG Vaishnav College, Chennai.

## RESULTS AND DISCUSSION

### **Visible Observation of Nano particle Synthesis**

The formation of silver nano particles was preliminarily confirmed by the change of the color of the solution. The color change from pale brown to dark brown color of the aqueous solution was due to the excitation of the surface plasmon resonance band<sup>12</sup> which plays an important role in the confirmation of the silver nano particle formation<sup>13</sup>. It also indicates the reduction of silver ion in the aqueous solution by the whole plant extract of *Marsilea minuta* L.

### **Characterization Studies**

#### **UV- Visible Spectrum Analysis**

Optimum intensity of UV-Visible spectra peak or SPR centered between 400-450 nm occurred and peak maximum at 437 nm which is attributed to the formation of silver nanoparticles for 1 and 3mM silver nitrate (Figure IA &B).

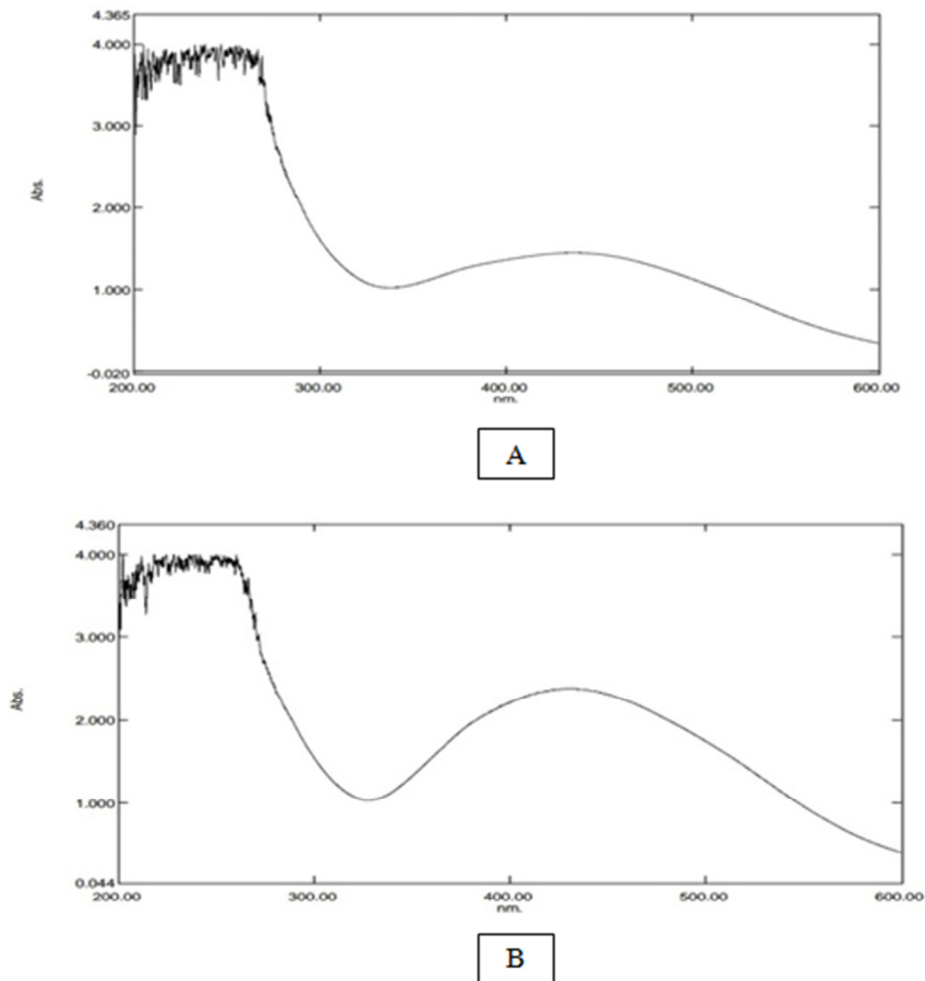


Figure 1 (A & B)  
 UV-Visible spectra recorded as a function of 1 and 3 mM AgNO<sub>3</sub> with aqueous extract of *Marsilea minuta* L. in a reaction at 37°C

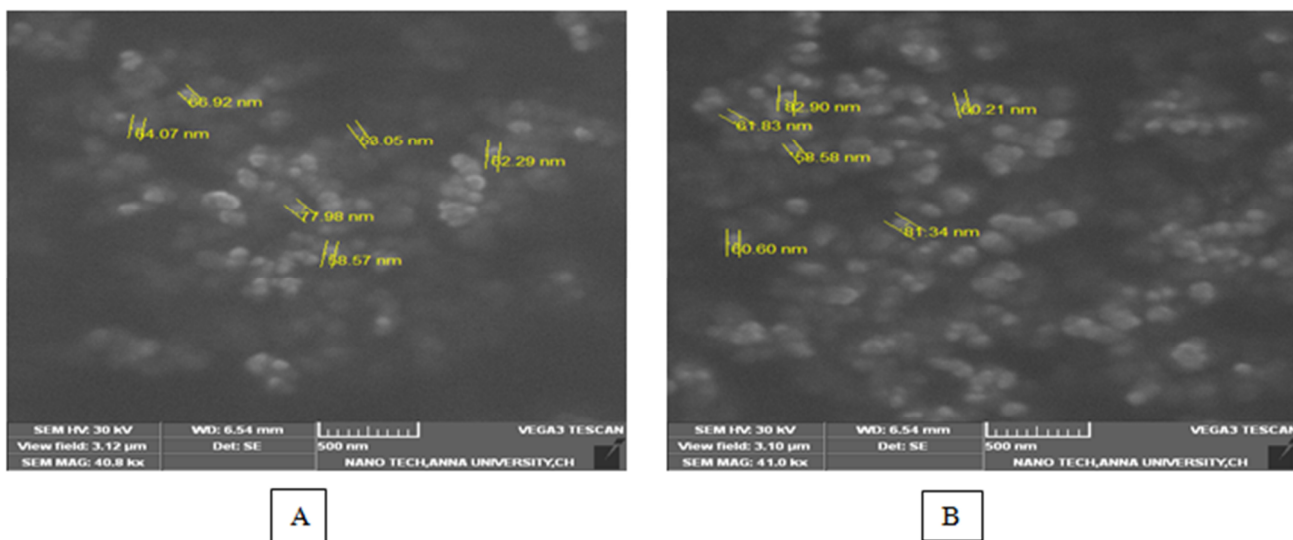


Figure 2 A & B  
 SEM analysis of a *Marsilea minuta* extract + 1 & 3mM AgNO<sub>3</sub> at 37°C

**SEM Analysis**

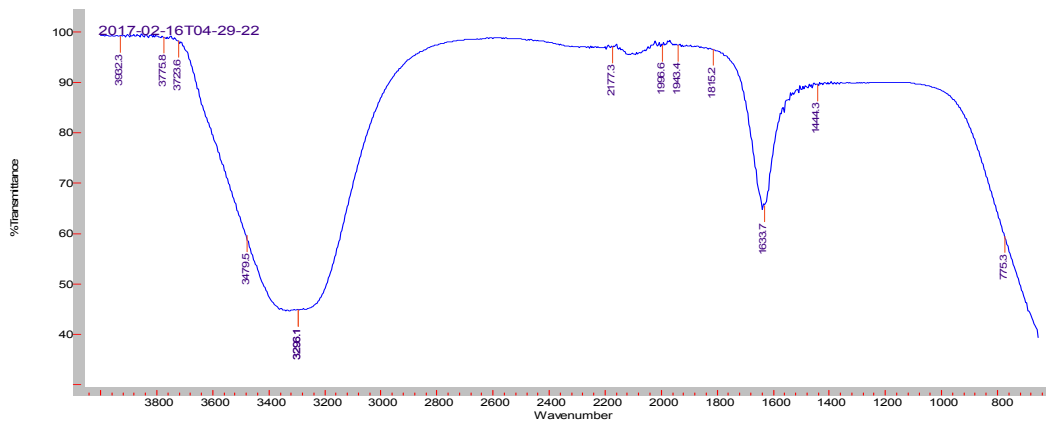
The size and shape of the biosynthesized nanoparticles were observed by SEM (Figure 2 A & B). The silver nanoparticles are spherical and near spherical in shape,

polydispersed and are slightly aggregated in solution. The size of the formed nanoparticles is found to be 53 - 95 nm.

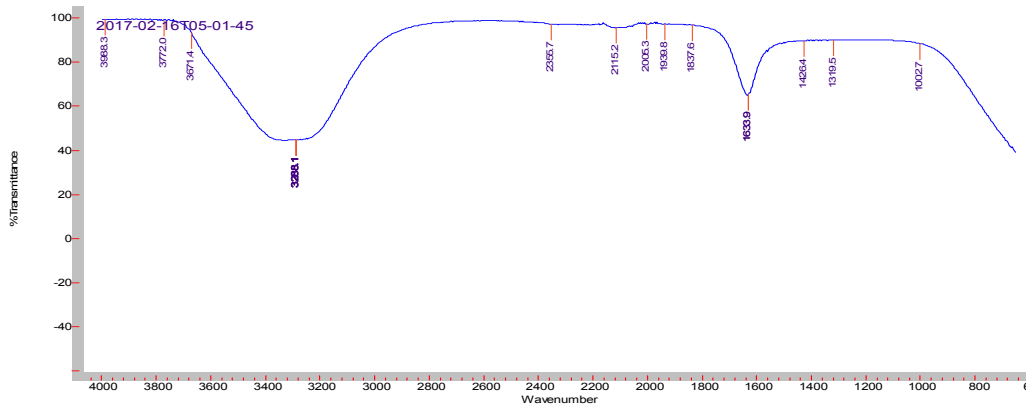
**FTIR**

The FTIR spectra were used to identify the possible bio-molecules responsible for the reduction of silver ions and capping of *M.minuta* formed AgNP. (Figure 3 A, B & C) shows the FTIR spectra of *M.minuta* aqueous extract, 1 & 3mM biosynthesized AgNP. In the FTIR spectrum *M.minuta* AgNPs were formed in pH 7 (inherent pH ), the additional transmission peaks for the plant extract containing Ag-NPs were at 3296,2116,

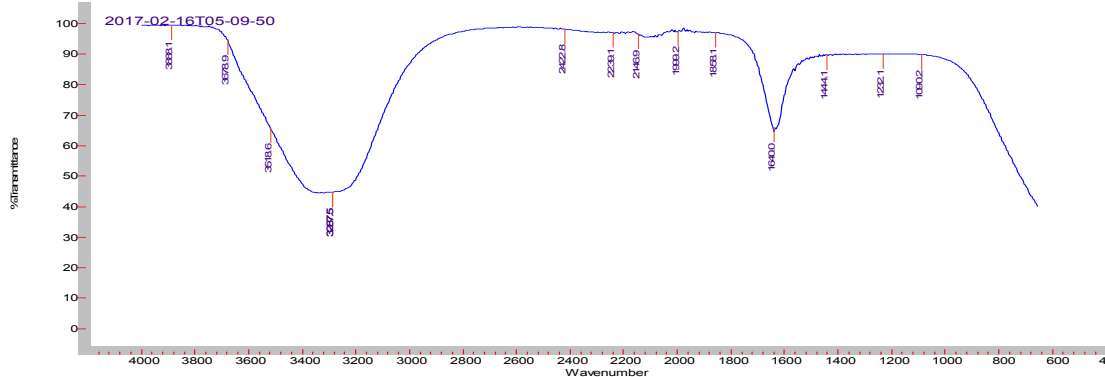
1634 & 1090  $\text{cm}^{-1}$  which corresponds to OH stretch (alcohols and phenols), C= C stretch (alkynes), NH bend(primary amines), C-O stretch (carboxylic acids, carbonyls, esters, aldehydes, saturated aliphatic) respectively. Therefore the FTIR results imply that AgNPs were successfully synthesized and capped with bio-compounds present in the *M.minuta* aqueous extract by using green method.



**Figure 3 A**  
FTIR analysis of *Marsilea minuta* aqueous extract



**Figure 3 B**  
FTIR analysis of *M. minuta* extract (pH7)+ 1mM  $\text{AgNO}_3$  at 37°C

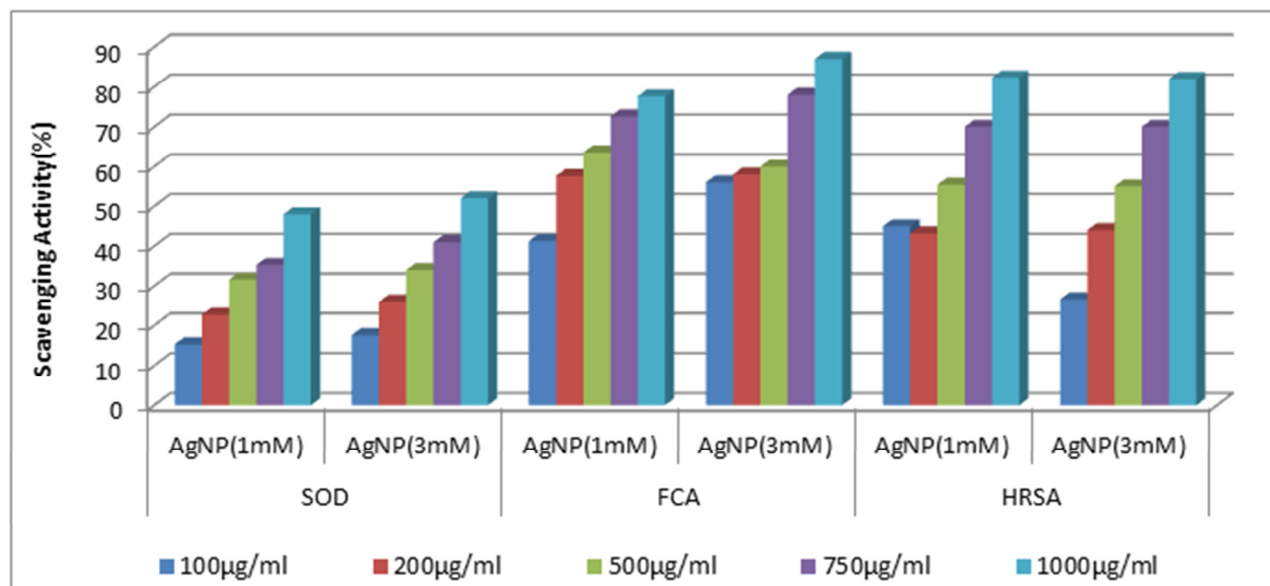


**Figure 3 C**  
FTIR analysis of *M.minuta* extract (pH7)+ 3mM  $\text{AgNO}_3$  at 37°C

**Antioxidant Activities**

The results of *invitro* antioxidant data showed a significant free radical scavenging activity of AgNPs (1& 3mM) in a dose dependent manner. The ferrous

chelating activity (FCA) was maximum in 3mM AgNPs (87.3%) with the IC<sub>50</sub> value less than 100µg followed by hydroxyl radical (82.6%) and SOD scavenging activities (52%) (Figure4).



**Figure 4**  
Antioxidant Scavenging Activity of AgNPs of *Marsilea minuta* Linn

**Antibacterial Activities**

The synthesized AgNPs inhibit the growth of micro organism. Growth inhibition zone values were obtained in mm for the synthesized Ag/*M.minuta* nanoparticle against five different microorganisms such as *Bacillus subtilis*, *Enterococcus faecalis*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. These were compared with positive control

(Streptomycin) and negative control (Dimethyl sulphoxide) (Table 1). The maximum inhibitory activity was seen in *B.subtilis*, *K.pneumoniae* and *E.faecalis* with inhibition zone of 12.6,13.3 & 13 mm respectively. 15µl AgNP of *M.minuta* shows more activity on *K.pneumoniae* and least effect on *B.subtilis*. 3mM AgNP of *M.minuta* shows more antibacterial activity when compared to the 1mM AgNP.

**Table 1**  
Antibacterial activity of synthesized AgNPs(1 & 3mM) of *Marsilea minuta*

S.no	Bacteria	Zone of Inhibition (mm)						Streptomycin
		1mMAgNP			3mMAgNP			
		5µl	10µl	15µl	5µl	10µl	15µl	
1.	<i>Bacillus subtilis</i>	6.9±0.5	8.6±0.47	10.3±0.5	10±0.8	12±0.8	12.6±0.62	19.2±0.5
2	<i>Klebseilla pneumoniae</i>	8.6± 0.47	10± 0.8	11.0± 0.8	10.3±0.47	12.3± 0.4	13.3± 0.5	18.3±0.6
3	<i>Staphylococcus aureus</i>	4.8±0.2	6.3±0.46	7.8±0.4	5.3±0.4	6.6±0.4	7.6±0.43	20.4±0.4
4	<i>Salmonella typhi</i>	4.5±0.4	46.6±0.47	7.0±0.8	5.3±0.9	6.8±0.23	7.3±0.5	15.2 ±0.5
5	<i>Enterococcus faecalis</i>	7.8 ±0.4	79.5 ±0.23	10.3 ±0.4	10.5 ±0.4	11.6±0.4	13 ±0.8	14.5± 0.7

Values were expressed as Mean ± SD (n=3) Standard (positive control): Streptomycin 10 µg/disc.

Panda et al reported that chloroform extract of *M.minuta* inhibits the growth of maximum pathogens compared to methanol extract. The highest zone of inhibition (25mm) was against *Staphylococcus citreus* in methanol extracts. with MIC value of 6mg/ml.<sup>14</sup> Poonam sethi has reported antibacterial activity of *M.quadrifolia* against the gram negative bacteria such as *Salmonella typhi*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Escherichia coli*<sup>15</sup> Synthesized silver nanoparticles of seaweed *Ulva lactuca* exhibited antibacterial and

antifungal activity<sup>16</sup>. The growth of microorganisms was inhibited by the green synthesized SNPs which may be due to the presence of peptidoglycon, which is a complex structure that contains teichoic acids or lipoteichoic acids which have a strong negative charge. This change may contribute to the sequestration of free silver ions<sup>17</sup>.

## CONCLUSION

*Marsilea minuta* L. leaf extract has shown the capability of biosynthesizing silver nano particles from Silver nitrate solution. The Ag-NPs were characterized by UV-Visible, SEM and FTIR measurements. The green chemistry approach addressed in the present work on the synthesis of silver nano particle is simple, cost effective, eco-friendly and the resultant nano particles are highly stable and reproducible. The synthesized Ag/NPs showed high antioxidant scavenging and anti bacterial activities.

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## CONFLICT OF INTEREST

Conflict of interest declared none.

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