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Green synthesis of silver nanoparticles from *p. canius* spine venom: characterization, antioxidant properties, and potential applications in nanomedicine

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Abstract

Venomous catfish of the order *Siluriformes* possess venom glands located in their dorsal and pectoral spines, which serve as a defense mechanism against predators. These fish, abundantly found in India's aquatic ecosystems, represent an untapped resource for bioactive compounds. Among them, *Plotosus canius*, a prominent species inhabiting the estuaries of southern India, produces venom with potential antioxidant properties. This study explores the antioxidant capacity of *P. canius* spine venom by isolating its protein components and utilizing the venom for the green synthesis of silver nanoparticles (AgNPs). The synthesized AgNPs were characterized using UV-visible spectroscopy, protein estimation, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). Their antioxidant activity was evaluated through the DPPH radical scavenging assay. The results indicate that *P. canius* venom demonstrates significant potential for the synthesis of functional nanoparticles with strong antioxidant properties, highlighting its applications in nanomedicine and environmental sustainability.

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INTRODUCTION

Nanotechnology, the manipulation of materials at the nanoscale, has revolutionized modern science and technology, offering innovative solutions across various fields, including medicine, environmental sustainability, and industry. Among the diverse nanomaterials, silver nanoparticles (AgNPs) stand out due to their unique properties, such as exceptional antioxidant activity, high surface-to-volume ratio, and remarkable optical and electrical behaviours. These attributes make AgNPs invaluable for applications in wound healing, drug delivery, and water purification [2]. However, conventional methods for synthesizing AgNPs rely predominantly on physical and chemical approaches, often involving toxic chemicals, significant energy consumption, and the

production of non-biodegradable by-products, which pose environmental and health risks [1]. To address these challenges, biological synthesis methods have emerged as an eco-friendly alternative, utilising natural resources for nanoparticle production [3].

Biological synthesis leverages the reducing and stabilising properties of biomolecules derived from natural resources. While venom extracts and microorganisms have been extensively studied for nanoparticle synthesis, animal-derived bioresources remain underexplored, despite their biochemical richness [19]. Among these, the freshwater catfish *Plotosus canius*, native to Indian and Southeast Asian waters, represents a promising and novel bioresource. Known for its venomous dorsal and pectoral spines, *P. canius* produces a venom rich in bioactive molecules, including enzymes, peptides, and toxins with significant biological activity [4]. These components act as natural reducing and capping agents, making the venom an ideal candidate for synthesising AgNPs.

The venom of *P. canius* offers dual functionality in nanoparticle synthesis: it facilitates the reduction of silver ions (Ag^+) into metallic silver (Ag^0) and stabilises the nanoparticles to prevent agglomeration. This approach aligns with sustainable development goals by minimising the use of harmful chemicals and energy-intensive processes. Moreover, the inherent bioactivity of venom-derived biomolecules imparts additional functional properties to the AgNPs, enhancing their potential applications in drug delivery and environmental remediation [18].

This study aims to synthesise AgNPs using *P. canius* venom and evaluate their physicochemical and biological properties. The nanoparticles were characterised using advanced techniques such as UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) to assess their structural, morphological, and functional attributes. Furthermore, their biological activity, particularly antioxidant potential, was evaluated using the DPPH radical scavenging assay [9]. This assay measures the nanoparticles' ability to donate electrons or hydrogen atoms, reducing the DPPH radical to a stable, non-radical form. AgNPs synthesised using *P. canius* venom are expected to exhibit significant free radical scavenging activity due to the bioactive molecules present in the venom. These antioxidant properties hold therapeutic potential, particularly in addressing oxidative stress-related disorders and enhancing biomedical applications.

The use of *P. canius* venom for AgNP synthesis not only broadens the scope of nanotechnology but also underscores the untapped potential of freshwater bioresources. By exploring the biochemical properties of this venom, this study contributes to the development of sustainable and eco-friendly nanoparticle synthesis methods, paving the way for future innovations in medicine and environmental science.

External Morphology

The body of the fish is long, sub-cylindrical, and eel-like, tapering and flattening near the tail region. It measures between 50–80 cm in total length, with a maximum size reaching up to 150 cm. The body is plain dusky-brown in colour, with darker fins. The upper region of the body and head exhibits a dark olive-green hue, while the ventral region is pale. The barbels and fins are gray in coloration, while the first dorsal and pectoral fins are darker and equipped with serrated spines identified as venomous.

The second dorsal fin is notably elongated and merges seamlessly with the caudal fin; similarly, the anal fin is also fused with the caudal fin. A fully developed lateral line is present, enhancing sensory perception, and a dendritic organ is located behind the ventral fin, near the genital pore [6]. The function of this organ, however, remains undetermined.

The head is large, broad, and depressed, covered with thick skin. The fish features diminutive ocular organs and possesses four pairs of elongated barbels, which include nasal, maxillary, and mandibular barbels [7]. These barbels contribute to the organism's sensory capabilities.

Materials and Methods

Collection and Processing of Sample

Live freshwater catfish were collected from the Kollidam River, situated along the southeast coast of India. The collected fish were stored at -2°C for 1 hour to maintain freshness. Dorsal and pectoral fins were carefully excised from the fish.

Subsequent processing steps, including homogenization, were conducted at 40°C. The excised fins were immediately transported to the laboratory for further analysis.

Methodology

Synthesis and Optimization of Nanoparticles

Silver nanoparticles (AgNPs) were synthesized using silver nitrate in combination with catfish spine powder. Initially, the spines were finely crushed into a powder. A 10-gram portion of this powdered sample was dissolved in 100 millilitres of double-distilled water (ddH_2O). The mixture was heated to 60°C for 20 minutes. Separately, a 0.01 M solution of silver nitrate was prepared by dissolving silver nitrate in ddH_2O.

Different ratios of silver nitrate to spine powder solution were tested, including 5:5, 6:4, 7:3, 8:2, and 9:1. Among these, the 8:2 ratio was selected for bulk nanoparticle synthesis, as it yielded the highest production efficiency. The reaction mixture was stirred continuously using a magnetic stirrer set at 800 rpm and heated just below the boiling point. Within an hour, the mixture developed a characteristic brown colour, indicating nanoparticle formation.

The entire synthesis process was conducted in complete darkness to prevent photo-induced reactions.The reaction mixture was centrifuged at 3,500 rpm for 10 minutes to collect the nanoparticles. The resulting pellet was washed three to four times with deionized water to remove any impurities. The purified silver nanoparticles were stored in a dark, dry, and cool environment for subsequent characterization.

Strategies for the Examination of Silver Nanoparticles (AgNPs)

Estimation of Protein

The protein content of the sample was determined using the method developed by Lowry et al., with bovine serum albumin (BSA) as the standard. Total protein concentration was measured spectrophotometrically at 640 nm, based on the established technique of Lowry et al. (1951).

UV-Visible Spectrophotometry

The synthesis of silver nanoparticles (AgNPs) was initially confirmed using UV-visible spectrophotometry (Perkin-Elmer, Lambda 35, Germany). The transformation of silver ions (Ag^+) into AgNPs was monitored by analyzing samples at regular time intervals. Absorbance spectra were recorded in the wavelength range of 200–800 nm, capturing surface plasmon resonance peaks characteristic of AgNP formation [5].

FTIR Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR, Perkin-Elmer) was used to identify the functional groups involved in the biological synthesis and stabilization of AgNPs. The FTIR spectra were recorded in the range of 400–4000 cm^{-1}, allowing for the assignment of specific vibrational modes to functional groups present in the catfish spine extract and the synthesized AgNPs. A comparative analysis of the spectra highlighted changes in functional groups, providing insights into their roles in nanoparticle synthesis and stabilization [8].

Evaluation of Antioxidant Activity

The antioxidant activity of the synthesized AgNPs was assessed using the DPPH radical scavenging assay, a widely used method for evaluating the antioxidant properties of natural products [MacDonald-Wicks et al., 2006]. The assay is based on the principle that antioxidants act as hydrogen donors [10]. The mechanism of the DPPH assay is illustrated in **Figure 1** , [11]. DPPH, a stable and commercially available organic nitrogen radical, serves as a standard for assessing antioxidant capacity. It has a prominent absorption peak at 517 nm, appearing purple in colour. Upon reaction with hydrogen atoms from an antioxidant, DPPH is reduced and transitions to a yellow hue. This stoichiometric reaction enables precise quantification of antioxidant activity. The reduction of DPPH, directly correlated to the antioxidant capacity of the sample, was measured using a UV spectrometer for its simplicity and high accuracy [12].

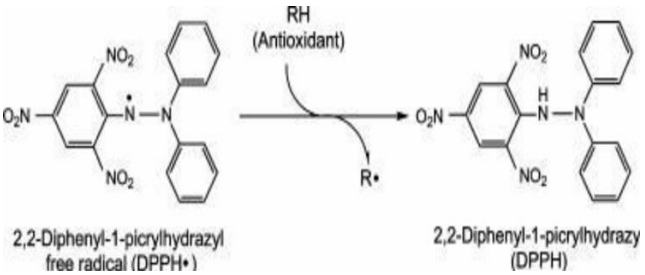

Figure 1 DPPH accepts hydrogen from an antioxidant

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was utilised to analyse the morphology and structural characteristics of the biosynthesised silver nanoparticles (AgNPs). Dried specimens were mounted onto a copper-coated carbon grid for observation. The analysis, conducted using a Hitachi S-4500 SEM, provided detailed insights into the shape and bonding configurations of the AgNPs [17].

Results

Characterization of AgNPs

UV-Visible Analysis

The UV-visible spectrum of the AgNPs displayed a distinct absorption band, indicative of silver nanoparticle formation. This band, located at a specific wavelength (nm), corresponds to the surface plasmon resonance phenomenon characteristic of AgNPs. The absence of additional peaks in this spectral range confirms the successful synthesis of pure silver nanoparticles (**Figure 2**). The observed spectral range is consistent with previously reported values for AgNPs, further validating the synthesis process.

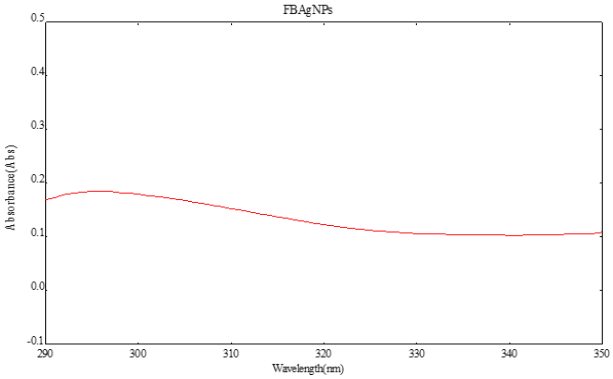

Figure 2 UV-visible

Preparation of Crude Extracts

The protein concentration data was determined using a standard protein concentration curve. This curve was generated by measuring the absorbance of bovine serum albumin (BSA) across a range of known concentrations. A line equation was derived from the plotted curve, serving as the basis for calculating the protein concentration of the sample (**Figure 3**). To determine the protein concentration, the absorbance value of the sample was measured and substituted into the standard curve equation. This process enabled the precise calculation of the protein concentration in the sample. The calculated protein concentrations for the various samples are summarised in **Table 1**.

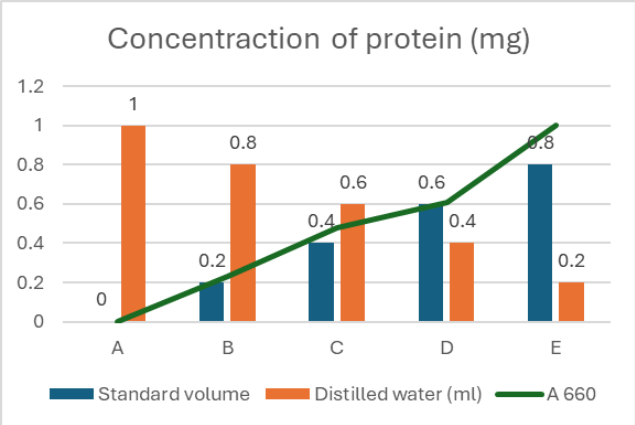

Figure 3 Concentraction of protein(mg) Bar Graph

FTIR Analysis

Fourier Transform Infrared (FTIR) spectroscopy was used to identify the functional groups involved in the synthesis and stabilization of silver nanoparticles (AgNPs). The spectra of both the crude extract and the AgNPs revealed distinct stretching vibration patterns of functional groups.

Table 1 Protein Estimation Data

SI.NO	Volume of standard	Volume of distilled water (ml)	Concentration of protein (mg)	Volume of reagent C (ml)	Incubate at room temperature for 10min	Volume of reagent D	Incubate at dark room temperature for 30min	A 660
1	0.0	1.0	00	4		0.4		0.00
2	0.2	0.8	40	4		0.4		0.23
3	0.4	0.6	80	4		0.4		0.48
4	0.6	0.4	120	4		0.4		0.61
5	0.8	0.2	160	4		0.4		1.00

A broad band observed at 3315 cm^{-1} was attributed to the stretching vibration of hydroxyl (OH) groups, while additional bands were noted at 611, 1088, 1387, 1638, 2312, 2856, 2930, and 3421 cm^{-1}.

Based on a review of the literature, functional groups such as phenolic, alcoholic, and carboxylic acid groups are implicated in the reduction of silver ions (Ag^{+}) and the stabilization of AgNPs. These chemical groups facilitate the biosynthesis process and prevent agglomeration of nanoparticles. The dried AgNPs were analysed using a Perkin Elmer FTIR spectrometer (USA), which provided detailed insights into the chemical composition and functional groups involved in the nanoparticle formation (**Figure 4**).

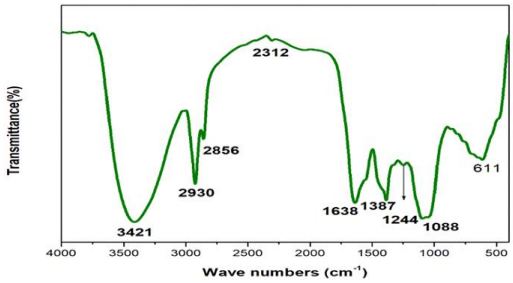

Figure 4 FTIR

DPPH Assay

The herbal extract of *P. canius* spine demonstrated a significantly higher concentration of reductants (antioxidants) compared to the corresponding methanolic extract. In general, an increase in absorbance value was directly correlated with an increase in extract concentration, indicating a positive trend in the extract's reducing power. This was reflected in the increasing optical density (OD) values measured at 517 nm. The control sample exhibited a mean OD value of 1.669 (**Figure 5**).

The antioxidant potential was further validated by the percentage of radical inhibition (**Figure 6**) and the IC_{50} value of the tested sample, calculated as 86.39 mg/ml (**Figure 7**). These results highlight the extract's capacity to act as an efficient radical scavenger. A methanolic solution of the stable free radical DPPH was employed to assess the scavenging ability of the extracts. The freshly prepared DPPH solution, characterised by its deep purple hue, underwent a colour change as it interacted with the antioxidants, confirming the extract's ability to neutralise free radicals.

OD Value at 517 nm

Control Mean OD value: 1.699

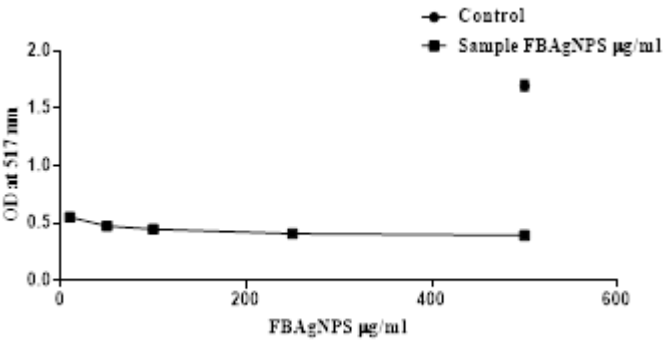

Figure 5 Control Mean OD value

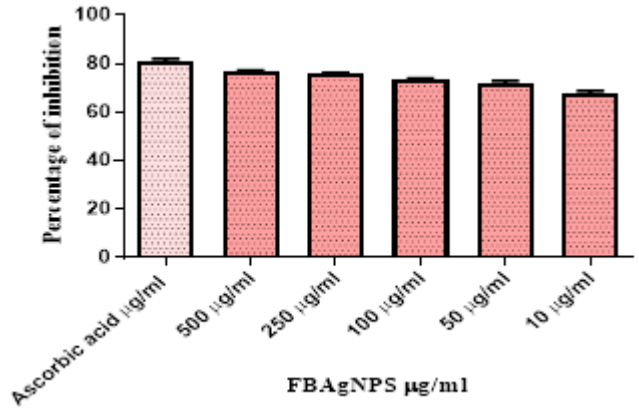

Figure 6 Percentage of inhibition

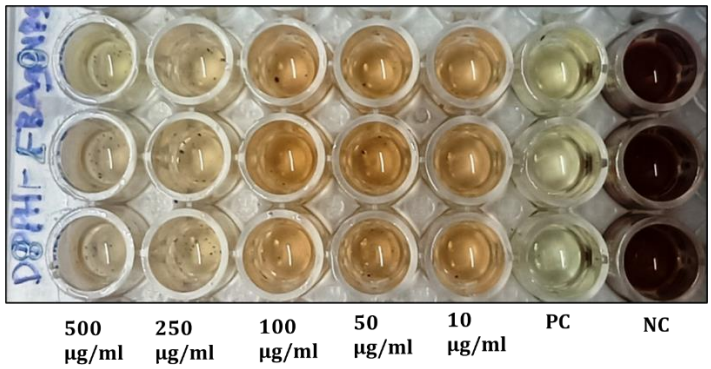

Figure 7 Shows the results of DPPH assay

SEM Assay

The morphology of biologically synthesized silver nanoparticles (AgNPs) using the methanolic extract of *P. canius* was observed through scanning electron microscopy (SEM). The SEM images (**Figure 8**) revealed nanoparticle aggregation, which could be attributed to solvent evaporation during sample preparation. This aggregation likely

contributed to the formation of larger AgNP particles and variations in particle size.

The elemental composition of the powdered materials was also assessed using a scanning electron microscope (JEOL-JSM6610LV). The analysis provided valuable insights into the structural and morphological characteristics of the synthesized nanoparticles, supporting the findings of the study.

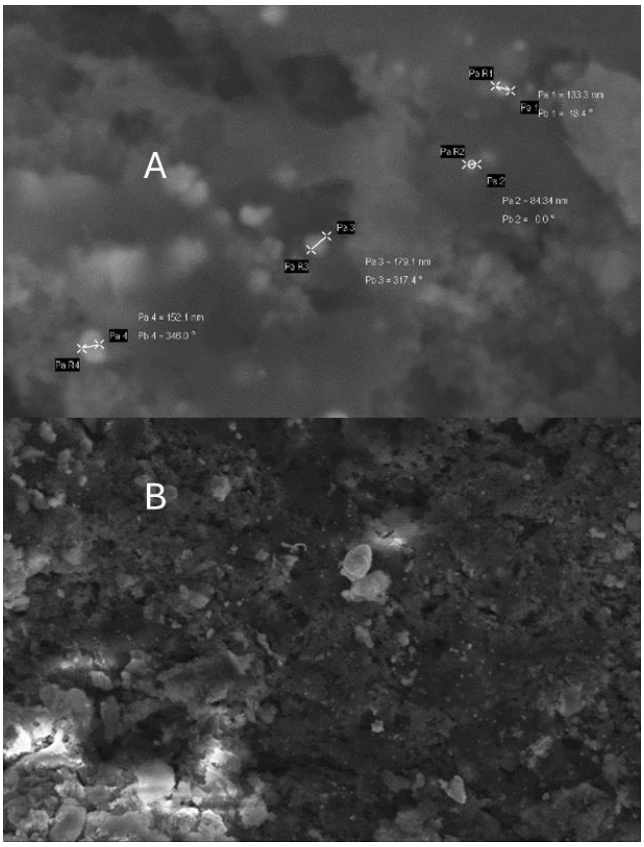

Figure 8 shows the results of SEM

Discussion

This study highlights the potential of using the venom of the freshwater catfish, *P. canius*, as a sustainable and eco-friendly resource for synthesizing silver nanoparticles (AgNPs). The successful synthesis of AgNPs was validated through multiple characterization techniques, including UV-visible spectroscopy, FTIR, and SEM, which collectively confirmed the effective reduction of silver ions and the stabilization of the resulting nanoparticles.

The UV-visible spectroscopic analysis revealed a prominent absorption peak characteristic of AgNPs, consistent with the surface plasmon resonance effect inherent to metallic nanoparticles [13]. The absence of extraneous peaks in the spectrum further confirmed the purity and specificity of the synthesized AgNPs, underscoring the effectiveness of the green synthesis approach employed in this study.

Protein estimation results demonstrated a significant concentration of proteins in the *P. canius* spine extract, which likely contributed to the reduction and stabilization processes during nanoparticle synthesis. The bioactive molecules, such as enzymes and peptides present in the venom, appear to play a critical role, as evidenced by FTIR analysis. The FTIR spectra revealed the presence of functional groups, including hydroxyl, phenolic, and carboxylic acid groups, which are known to facilitate the reduction of metal ions and stabilize nanoparticles [14], [15]. This underscores the biochemical complexity of the venom and its dual role in both reducing and stabilizing nanoparticles.

The antioxidant activity of the synthesized AgNPs, assessed via the DPPH assay, demonstrated significant radical scavenging capabilities. With an IC_{50} value of 58.22 µg/ml, the AgNPs exhibited strong antioxidant properties, likely due to the bioactive compounds present in the venom. These findings align with previous studies that report similar antioxidant activities in nanoparticles synthesized through biological methods [16]. The free radical scavenging potential of these nanoparticles indicates their applicability in therapeutic contexts, particularly for managing oxidative stress-related disorders.

SEM analysis provided valuable insights into the morphology of the synthesized AgNPs, revealing particle size variations, potentially due to aggregation during the drying process. Such aggregation is a common challenge in nanoparticle synthesis and suggests opportunities for further optimization of synthesis parameters to achieve more uniform particle sizes. Uniformity in particle size is critical for enhancing the biological efficacy and stability of AgNPs in various applications [17].

The exploration of *P. canius* venom as a biogenic resource for AgNP synthesis presents new possibilities in the fields of nanotechnology and materials science. This study not only enhances our understanding of the biochemical properties of catfish venom but also underscores the potential of environmentally friendly methods for synthesizing

nanoparticles. Future research should focus on optimizing synthesis conditions to improve nanoparticle uniformity and exploring the broader biomedical applications of these AgNPs, particularly in drug delivery systems and wound healing, given their demonstrated antioxidant activity.

Conclusion

The successful synthesis of silver nanoparticles (AgNPs) using *P. canius* venom highlights the potential of natural resources for innovative and sustainable nanomaterial production. This eco-friendly approach not only aligns with sustainable development goals but also underscores the largely untapped potential of aquatic biomes in advancing nanotechnology, with promising applications in medicine and environmental science.

Comprehensive characterization of the synthesized AgNPs was achieved using UV-visible spectroscopy, FTIR, and SEM, demonstrating their effective synthesis and stability. The synthesized nanoparticles exhibited significant potential against pathogenic microorganisms, paving the way for their utilisation in biomedical applications. Additionally, the antioxidant activity of these AgNPs, as assessed by the DPPH assay, further underscores their therapeutic potential.

Future studies are essential to investigate the detailed mechanisms of action and therapeutic benefits of these AgNPs in biological systems. Moreover, further evaluation of their in vitro antioxidant activity and clinical efficacy is necessary before they can be considered for widespread medical use. It is envisaged that these Ag-doped nanoparticles could be effectively employed in drug delivery systems and other pharmaceutical applications, contributing to advancements in nanomedicine

Ethical Approval

No ethical approval was necessary for this study.

Author Contribution

All authors significantly contributed to laboratory activities; including plant extract preparation, FTIR and GCMS analysis, manuscript preparation, and tabulation. They consented to submit to the current journal, provided final approval for the version to be published and accepted accountability for all aspects of the work. All authors meet the eligibility criteria established by the International Committee of Medical Journal Editors.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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