GESA
GLOCAL ENVIRONMENT & SOCIAL ASSOCIATION

International Journal of Biological Innovations
http://ijbi.org.in | http://www.gesa.org.in/journals.php
https://doi.org/10.46505/IJBI.2025.7210
IJBI 7(2): 186-193 **(2025)**
E-ISSN: 2582-1032

APPLICATION OF DNA BARCODING FOR AUTHENTICATION OF SOME COMMERCIAL *ALOE VERA* PLANT POWDER USING matK GENE AS A BARCODE

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Article Info:
Research article
Received
10.06.2025
Reviewed
20.07.2025
Accepted
05.08.2025

Abstract: Medicinal plants hold significant importance in both traditional and modern healthcare practices, providing a wide range of therapeutic benefits. Among them, *Aloe vera* is highly valued for its wide range of pharmacological properties and commercial applications. India is a leading producer and exporter of *Aloe vera* and other herbal plants, particularly within the Asia-Pacific market. *Aloe vera* is used extensively in pharmaceutical, cosmetic, food, and nutraceutical industries. However, the authenticity of commercial *Aloe vera* powder remains a concern due to possible adulteration, especially in processed forms where morphological identification is ineffective. This raises serious issues related to consumer safety, product integrity, and trade reliability. DNA barcoding has emerged as a reliable molecular technique for accurate species identification, even in degraded or powdered samples. In this study, DNA barcoding is applied using matK (Maturase K) gene as a barcode to authenticate 11 *Aloe vera* powder samples collected from various districts of West Bengal. The results aim to highlight the effectiveness of this approach in detecting adulteration and ensuring product quality in the herbal industry.

Keywords: Adulteration, *Aloe vera* plant powder, DNA barcoding, matK, Phylogenetic tree.

Cite this article as: Ahamed Z., Mallick B., Panigrahi A.K. and Mandal S.K. (2025). Application of DNA Barcoding for authentication of some commercial *Aloe vera* plant powder using matK gene as a Barcode. *International Journal of Biological Innovations*. 7(2): 186-193. https://doi.org/10.46505/IJBI.2025.7210

INTRODUCTION

Medicinal plants play a vital and key role in both traditional healing practices and modern healthcare systems, providing remedies for a wide range of health conditions (Miranda, 2021). Several Indian texts including the Vedas document their early use, while the Ayurveda have relied on them for over 5,000 years (Tucakov, 1971; Morgan, 2002). The natural origin of the medicinal plants and historical significance contribute to their widespread use, often without concerns about safety (Yadav and Dixit, 2008; Rao, 2021; Singh, 2023). With increasing global demand, particularly for powdered herbal products, nearly 80% of the global population now depends on plant-based remedies for

primary healthcare (McKenzie *et al.*, 2009). India plays a central role in this sector, utilising around 8,000 species for therapeutic purposes and trading approximately 960 species commercially (Mishra *et al.*, 2015; Srivastava *et al.*, 2016; Sharma and Pareek, 2021).

Among these, *Aloe vera* is one of the most widely recognised medicinal plants, valued for both its therapeutic efficacy and economic potential. It contains over 75 biologically active compounds including acemannan, aloin, aloe-emodin, polysaccharides, enzymes, and vitamins, which are linked to its antioxidant, anti-inflammatory, antimicrobial, antidiabetic, wound-healing, and immune-modulatory properties (Maan *et al.*, 2018). *Aloe vera* is very effective for treating arthritis, diabetes, and certain skin conditions (Radha and Laxmipriya, 2014; Saleem *et al.*, 2022). The versatile applications of *Aloe vera* across pharmaceutical, cosmetic, food, and nutraceutical industries have made it a high-value commercial crop. India, with its extensive cultivation and processing infrastructure, has become a major contributor to the growing Asia-Pacific *Aloe vera* market (Kumar *et al.*, 2022; Sharma and Kaur, 2025).

Despite its popularity, ensuring the authenticity of *Aloe vera* powder is still a major challenge, as traditional morphological identification is not so effective for the processed samples. Adulteration, whether intentional or unintentional, not only compromises product efficacy but also poses risks to consumer safety and the credibility of India's herbal exports (Sahoo and Manchikanti, 2013; Mishra *et al.*, 2015; Kumar *et al.*, 2018). In this context, DNA barcoding has proven to be a powerful tool for accurate species identification, even in degraded or powdered materials (Vassou *et al.*, 2016; Das and Joshi, 2023). The present study utilises DNA barcoding to authenticate 11 commercially available *Aloe vera* powder samples sourced from different districts in West Bengal, aiming to assess product integrity and ensure consumer safety.

Table 1: District wise collection details of 11 *Aloe vera* powder.

S. No.	Powder code	Collection details (District)
1.	S1	Nadia
2.	S2	Murshidabad
3.	S3	Purba Bardhaman
4.	S4	Hooghly
5.	S5	Kolkata
6.	S6	North 24 Parganas
7.	S7	Purba Medinipur
8.	S8	Bankura
9.	S9	Malda
10.	S10	Uttar Dinajpur
11.	S11	Cooch Behar

MATERIALS AND METHODS

Collection details of *Aloe vera* plant and *Aloe vera* plant powder

Economically important *Aloe vera* plant (T1) was collected from Haringhata, W.B. (22.9629760, 88.6265670) and 11 *Aloe vera* plant powder (S1 to S11) were purchased from different places of West Bengal. Sample collection details of 11 *Aloe vera* plant powders are presented in Fig 1 and Table 1. The map shape file was obtained from the online maps portal of the Survey of India (2025) and processed using ArcGIS (2025) software for map construction (Fig 1).

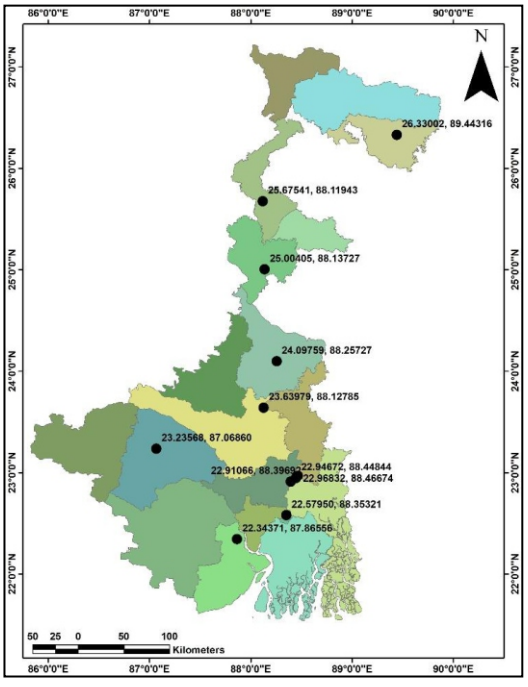

Fig. 1: Collection details of 11 *Aloe vera* plant powder (using ArcGIS software).

Isolation of Genomic DNA and Selection of Primers

Genomic DNA of the *Aloe vera* plant and *Aloe vera* plant powder sample was isolated using DNeasy® Plant Mini Kits (2025) (Catalogue Number: 69104) and the isolation steps are presented in Fig 2. Agarose gel electrophoresis was done for the quality check of the isolated genomic DNA.

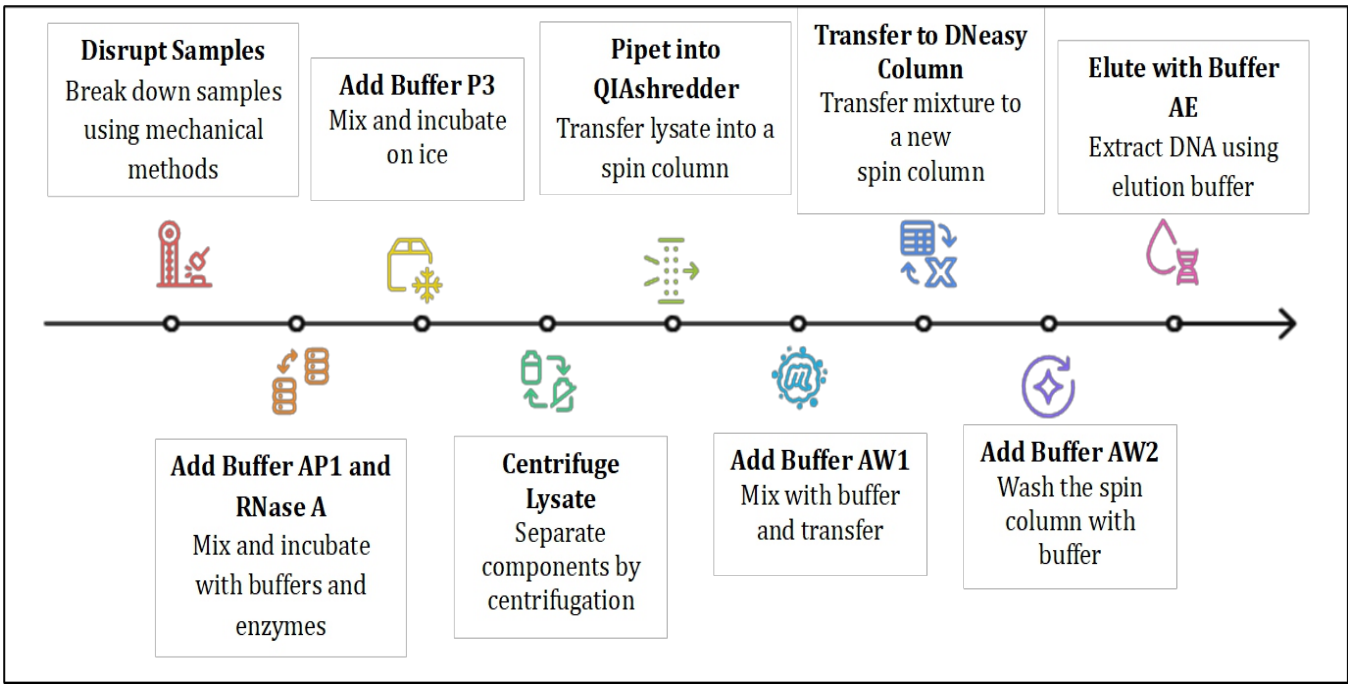

Fig 2: Genomic DNA isolation process according to the kit protocol.

Among the high value medicinal plants, *Aloe vera* is one of the most important medicinal plant species. According to Consortium for the Barcode of Life (CBOL) Plant Working Group, Maturase K gene matK is considered as a plant identification marker for DNA barcoding of medicinal plants (Hollingsworth *et al.*, 2009). Forward primer 5' -TAATTTACGATCAA TTCATTC- 3' and Reverse primer 5' -ACAAGAAAGTCGAAGTAT- 3' were used for PCR (Polymerase Chain Reaction) with 42°C annealing temperature (Algarni, 2021).

PCR, Post PCR Cleanup and Sequencing of DNA

MiniAmp™ Plus Thermal Cycler (2025) (Catalogue Number: A37835) was used for PCR and the reaction steps are presented in Fig 3.

Table 2: Reaction mixture of Polymerase Chain Reaction (PCR).

S. No.	Components	Volume (µl) (for 50 µl reaction)
1.	GoTaq® 2X Green Master Mixes	25
2.	Forward Primer (20 µM)	5
3.	Reverse Primer (20 µM)	5
4.	Genomic DNA	10
5.	Nuclease Free Water	5

Promega ReliaPrep™ DNA Clean-up and Concentration system (2025) (Catalogue Number: A2892) was

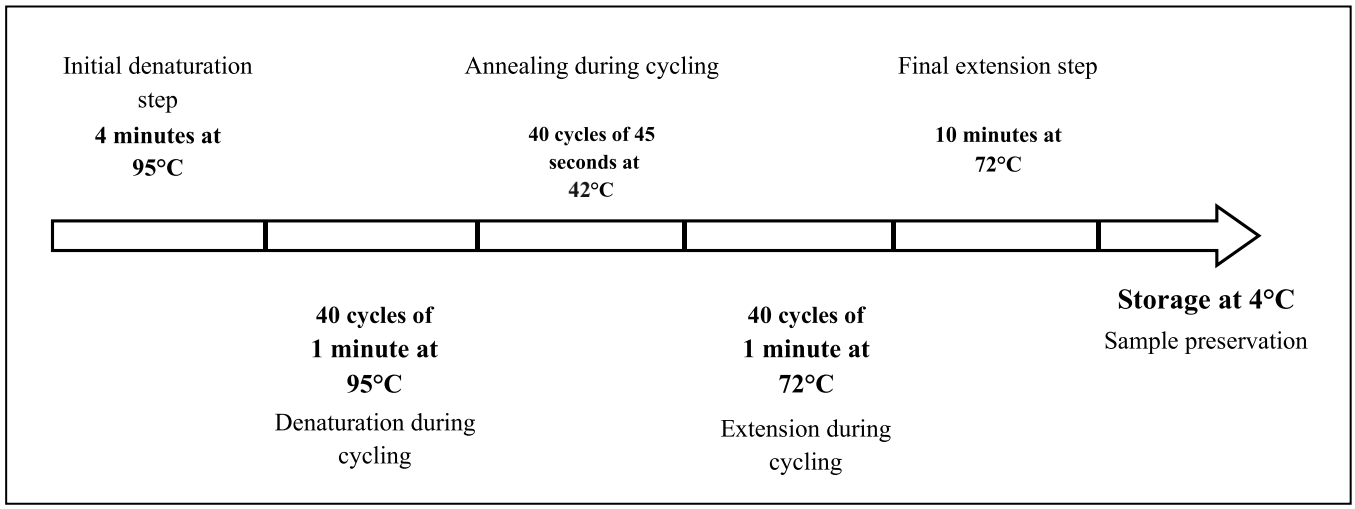

Fig. 3: Flow chart of Thermal profile of PCR reaction.

used for the purification of PCR product. The purified PCR product was labelled by using BigDye™ Terminator v3.1 Cycle Sequencing Kit (2025) (Catalogue Number: 4337455) and Applied Biosystems® 3500 Series Genetic Analyzer (2025) by Thermo Fisher Scientific was used to obtain DNA sequences.

Analysis of Sequence Chromatogram

The sequencing data of *Aloe vera* plant and powder samples were obtained and the chromatogram data analysis was done by using BioEdit (2025) software. Then the FASTA sequences were extracted for phylogenetic analysis. The matK barcode gene sequence of *Aloe vera* is used as reference DNA barcode.

Species Authentication through Phylogenetic Analysis using NCBI Database

Phylogenetic tree was constructed using the FASTA sequence of 11 powder samples and the matK sequence (FASTA sequence of matK) of *Aloe vera* plant. To determine the authenticity of the powdered samples of *Aloe vera*, a detailed phylogenetic analysis was conducted. The assessment focused on the clustering behaviour of each sample within a constructed phylogenetic tree, which allowed for the visualization of genetic relationships. This tree was generated using the Neighbour-Joining (NJ) algorithm implemented in MEGA software (Kumar *et al.*, 2016), a widely recognized tool for molecular evolutionary analysis. Genetic distance among sequences were calculated using the Kimura 2-Parameter (K2P) model, which is well-suited for evaluating evolutionary divergence in DNA barcoding studies (Kimura, 1980; Tamura *et al.*, 2021). To ensure the robustness and reliability of the resulting tree topology, a bootstrap analysis with 1000 replicates was applied, providing statistical support for each branch.

Furthermore, in order to confirm species-level identification, the DNA barcode sequences obtained from the suspect samples were aligned and compared against universally available reference sequences in the NCBI GenBank database (NCBI, 2025). This was achieved through the Basic Local Alignment Search Tool (BLAST), which identifies close genetic matches based on sequence similarity. By integrating clustering patterns from phylogenetic analysis with sequence alignment data from BLAST, a comprehensive and accurate approach to sample authentication was established.

RESULTS AND DISCUSSION

Genomic DNA Isolation, PCR Amplification and DNA Sequencing

Genomic DNA isolation from all the 11 samples was done successfully. PCR amplification with matK gene and DNA sequencing of all the amplified matK barcode were done with 100%

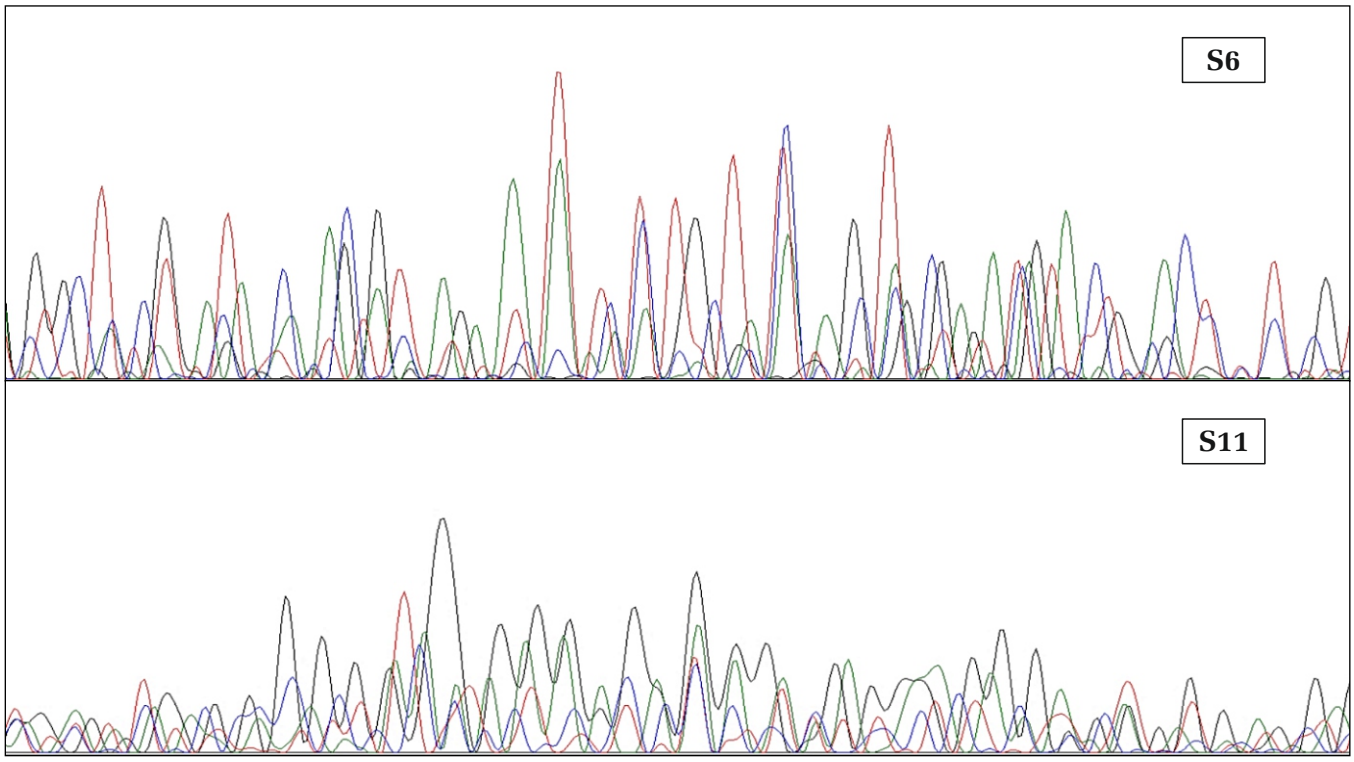

Fig 4: Chromatograms of mixed samples (S6 and S11) showing unreadable DNA sequences.

success rate. The chromatograms of 2 powder samples were completely unreadable, containing multiple overlapping regions. The overlapping regions are the clear indication of multiple species mixture which contributes to the multiple DNA fragments within the same powder (Fig. 4). Therefore, S6 and S11 are categorized as mixed powder samples.

In cases where DNA is isolated from mixed powder samples, the resulting sequences are typically unsuitable for direct analysis due to the presence of genetic material from multiple species, which makes it difficult to resolve individual components using standard DNA barcoding methods. As a result, identifying adulterant species through conventional barcoding becomes impractical. To overcome this limitation, advanced molecular approaches such

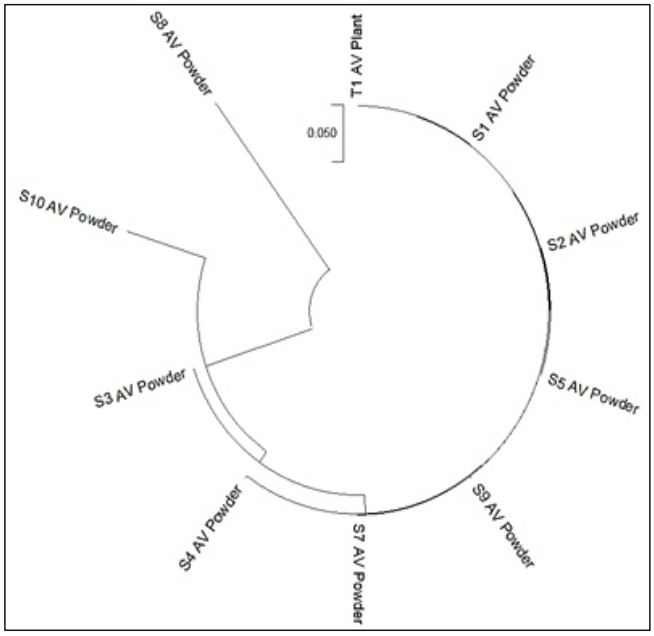

Fig 5: Phylogenetic tree of 11 *Aloe vera* plant powder samples with reference DNA Barcode.

Table 3: Species identification of non-authentic *Aloe vera* powder samples using NCBI database.

S. No.	Powder Sample Code	Barcode Gene	Expected Species as per label	Species identified by DNA Barcoding (using NCBI database)	NCBI Accession Number
01	S3	matK	*Aloe vera*	*Haworthiopsis attenuata*	KJ557841.1
02	S8			*Echinocereus grandis*	FN997344.1
03	S10			*Aloe aculeata*	KJ557717.1

as DNA metabarcoding, which enables simultaneous detection of multiple species within a complex sample (Seethapathy *et al*., 2019; Urumarudappa *et al*., 2020) or cloning of DNA barcode amplicons followed by sequencing are employed to accurately determine the species composition of such mixed powder samples (Spooner, 2009).

Species Authentication using Phylogenetic Tree Analysis and NCBI Database

Phylogenetic analysis of 9 readable *Aloe vera* powder sequences was done to authenticate their species. With reference to the NCBI database and the phylogenetic tree (Fig. 5), constructed using sequences from the reference DNA barcode and the *Aloe vera* powder samples, 6 samples (S1, S2, S4, S5, S7, S9) were found to be authentic and the rest 3 samples (S3, S8, S10) were non-authentic (Table 3). Among the 3 non-authentic samples, 1 sample (S10) was substituted with a species from the same genus, whereas the remaining 2 samples (S3, S8) were replaced with species from entirely different genera.

The present study revealed that *Aloe vera* powders which were collected from Nadia (S1), Murshidabad (S2), Hooghly (S4), Kolkata (S5), Purba Medinipur (S7) and Malda (S9) district are found to be authentic. The *Aloe vera* powders collected from Purba Bardhaman (S3), Bankura (S8) and Uttar Dinajpur (S10) are non-authentic. The *Aloe vera* powder S3 is substituted by the species *Haworthiopsis attenuata,* powder S8 is substituted by *Echinocereus grandis* and the powder S10 is substituted with same genus but different species *Aloe aculeata.*

CONCLUSION

DNA barcoding has emerged as a reliable and promising method for authenticating medicinal plant powders and identifying plant species. In this study, high-quality genomic DNA was successfully extracted at various concentrations using a commercial kit-based method. The obtained results demonstrated that PCR amplification and sequencing of the matK region of DNA effectively identified *Aloe vera* plant powder samples. Among the 11 samples analysed, 6 were confirmed as authentic, 2 were identified as mixed samples, and 3 were found to be non-authentic. Among the non-authentic samples, 1 sample was replaced with a different species within the same genus, while the other 2 samples were substituted with completely unrelated plant species.

CONFLICT OF INTEREST

The authors declare that they have no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Ecological Studies and International Centre for Ecological Engineering (ICEE) as well as Department of Food and Nutrition, University of Kalyani, Kalyani, Nadia (West Bengal), India for providing the laboratory support. The authors are also thankful to Mrs. Ziniya Banu Mallick, Department of Physiology, University of Calcutta (W.B.) for mapping collection area using ArcGIS Software and Mr. Sabyasachi Bhattacharya, of ICEE for his sincere help to prepare this manuscript. One of the authors (ZA) also acknowledges the Department of Higher Education, Government of West Bengal for providing him Swami Vivekananda Merit Cum Means (SVMCM) fellowship to carry out the research work.

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