Research Article

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Dye degradation activity of biogenically synthesized Cu/Fe/Ag trimetallic nanoparticles

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Abstract: Over the past few decades, nanotechnology has gained momentum because of its potential to create a safer and healthier living environment using eco-friendly approaches. This study describes a safer, more dependable, and ecologically friendly technique of biologically synthesizing Cu/Fe/Ag trimetallic nanoparticles (NPs) using an aqueous leaf extract of Catharanthus roseus as the reducing and stabilizing agent. The synthesized trimetallic NPs were characterized by scanning electron microscope, Fourier transform infrared, dynamic light scattering, and ultraviolet-visible analysis and were evaluated for their potential applications, which included antioxidant properties and catalytic dye degradation. The result suggests that the antioxidant properties of Cu/Fe/Ag NPs are more significant than those of ascorbic acid, a known antioxidant, at lower doses (10 µg·ml⁻¹) while a higher dose of 1,000 µg·ml⁻¹ gives a 69.81% scavenging activity. The impact of Cu/Fe/Ag trimetallic NPs on the catalytic degradation of hazardous dyes such as phenol red (PR) and eosin yellow (EY) was also studied in this work. For PR and EY, the corresponding percentages of degradation were 76% and 48.6%, respectively.

Keywords: trimetallic nanoparticles, green synthesis, antioxidant activity, catalytic dye degradation

1 Introduction

Recent research has placed significant emphasis on metal nanoparticles (NPs) due to their beneficial biological properties, non-harmful nature, and exceptional characteristics [1]. These properties make metal NPs to be more desirable for different types of applications. For instance, silver NPs were synthesized using Lippia nodiflora aerial extract, Mimusops elengi fruit extract, cocoa pod shells, Rheum ribes, and the NPs were found to possess good antioxidant, antibacterial, cytotoxic effects, and antibiofilm activity nanocatalysts, antifungal agents, anticarcinogenic, and enzymatic activity [2–6]. In another study, palladium NPs and ZnO nanoflowers were fabricated in an environmentally friendly manner, and the NPs were found to be very good anticancer, antimicrobial, DNA cleavage, antioxidant activities, and photocatalytic removal of methylene blue dye under natural solar radiation [7,8]. Another study reported synthesis of Ag–CuO, Ag–Zn, Ag–Fe₂O₃, and Ag–Pt bimetallic NPs using orange, kiwi, and cabbage peel extracts and propolis extract and investigated their antibacterial, dye degradation applications and catalysis for hydrogen generation technology, respectively [9–12]. However, these NPs are known to have some limitations such as reusability and poor recovery. To overcome these limitations, scientists have been conducting numerous research including supporting the metal NPs on solid supports like polymer [13] or blending different types of metals to create hybrid metallic NPs like bimetallic or trimetallic NPs [14].

Trimetallic NPs refer to nanoscale particles composed of three different types of metals. These NPs typically exhibit unique properties due to the combination of multiple metals, which can interact synergistically to enhance various functionalities such as catalytic activity, electrical...
conductivity, optical properties, and magnetic behavior. The structure of trimetallic NPs often features a core made of one metal, surrounded by an interlayer of another metal, and further covered by a third metal. This “triple core–shell” architecture contributes to the distinct characteristics and applications of these NPs. Trimetallic NPs have garnered significant attention in recent research due to their potential in diverse fields, including catalysis, sensing, biomedicine, and environmental remediation. Numerous techniques can be used to fabricate trimetallic NPs, including physical and chemical methods [14,15]. However, a new area of study in the creation of trimetallic NPs from biological sources has significantly emerged. Trimetallic NPs can be produced using biological sources, including plant extracts, bacteria, fungi, and algae, since they are non-toxic, environmentally friendly, and scalable [16]. The biological method is considered a green synthesis and preferable to physical and chemical methods because they produce uneven particle size and involve using toxic chemicals that have a negative impact on the environment. Research has documented the effective synthesis of trimetallic NPs from biological sources in the literature. For instance, an article described the creation of Bi–Fe–Sn trimetallic NPs and their potential uses in medicine utilizing an extract from the leaves of Terminalia arjuna [17]. All things considered, the synthesis of trimetallic NPs from biological sources is a feasible strategy for the creation of reasonably priced and environmentally friendly nanomaterials that have potential uses in a variety of industries, including energy, environmental remediation, and medicine.

Catharanthus roseus, commonly known as the “Madagascar periwinkle” or “rosy periwinkle,” is a flowering plant native to Madagascar but cultivated worldwide for its ornamental value and medicinal properties. The plant is home to over 130 alkaloids, many of which have medicinal qualities and were employed in traditional medicine to treat a range of conditions. It belongs to the Apocynaceae family and is characterized by its glossy green leaves and vibrant flowers, which can range in color from white to pink to purple. Beyond its aesthetic appeal, C. roseus is renowned for its pharmacological significance [18]. It contains various alkaloids, including vincristine and vinblastine, which have potent anticancer [19], antioxidant [20], antidiabetic, and antimicrobial [21] properties. These alkaloids are used in chemotherapy to treat various types of cancer, including leukemia, lymphoma, and solid tumors. Additionally, C. roseus has been investigated for its potential in treating diabetes, hypertension, and microbial infections due to its bioactive compounds [22]. In traditional medicine, extracts from C. roseus have been utilized for their antidiabetic, antihypertensive, and antimicrobial properties. The plant’s alkaloids have been the subject of extensive research and are synthesized to develop pharmaceutical drugs for cancer treatment. Overall, C. roseus is valued not only for its beauty but also for its significant contributions to medicine, particularly in the treatment of cancer and other diseases.

Because of these reasons, we explore the synthesis of trimetallic NPs of Cu, Fe, and Ag using C. roseus leaf extract as the reducing and stabilizing agent for antioxidant and dye degradation application. The active phytochemical content present in the C. roseus leaf extract serves as both the reducing and stabilizing agent for the trimetallic Cu/Fe/Ag NPs.

2 Materials and methods

C. roseus leaves were collected from locally grown plants. Silver nitrate (AgNO3) (Qualigens, 99.9% purity), Mol weight = 169.87; ferrous sulfate heptahydrate (FeSO4·7H2O), (SRL, 98% purity), Mol weight = 278.01; and cupric sulfate pentahydrate (CuSO4·5H2O) (SRL, 99.5% purity), Mol weight = 249.68 were obtained from the lab. 11-Diphenyl-2-picrylhydrazyl (DPPH) (SRL, 99% purity), Mol weight = 394.32, and L-ascorbic acid (C6H8O6) (99% pure, SRL), Mol weight = 176.12, were obtained from the laboratory of Department of Biotechnology, Sharda University. Additionally, ethanol (Merck, 99% purity) was obtained from the laboratory and utilized as a solvent in the DPPH experiment. Distilled water was used throughout the experiment.

2.1 Preparation of the extract

All contaminants and dust were removed from the surface of the C. roseus leaves by gathering, cleaning, and disinfecting them with Labolin before using distilled water. The leaves were dried in a hot air oven at 50–60°C and then ground into a fine powder using a mortar and pestle. A 250 ml beaker was filled with 5 g of high-quality C. roseus and 100 ml of distilled water. For 1 h, the mixture was kept at 50°C in a water bath. After cooling, the mixture was filtered using Whatman’s filter paper No. 1, and the extract was packed in an airtight container in preparation for future use in the production of NPs.

2.2 Preparation of salt solutions

To make a 10 mM stock solution of the metal salts in a conical flask, 0.27801 g of FeSO4·7H2O, 0.2496 g of CuSO4·5H2O, and 0.1698 g of AgNO3 were dissolved in distilled water at room temperature to form 100 ml of solution. The stock solution was stored in a refrigerator for later use.
2.3 Fabrication of trimetallic NPs using *C. roseus* extract

To fabricate the trimetallic NPs, equal volumes of salt solutions containing Ag, Cu, and Fe were mixed together in a 250 ml beaker, and 20 ml of *C. roseus* aqueous leaf extract was added to the mixture of the metal precursors at a ratio of 1:1:1:1. The final mixture was then kept in a shaker incubator overnight at 50°C to produce a homogeneous combination. As a result, a dark greenish tint developed, signifying the synthesis of trimetallic NPs. It is reasonable to believe that the phytochemical components of the extract carried out the bioreduction of Ag$^+$ to Ag NPs, Cu$^{2+}$ to Cu NP, and Fe$^{2+}$ to Fe NPs because no additional reducing agent was added (Figure 1). In order to obtain powdered NPs, an aqueous solution of the synthesized trimetallic NPs was poured into a Petri dish and heated at 100°C for an hour in a hot air oven until completely evaporated. The solid product was then scraped off of the glass petri dish and stored in an Eppendorf tube for further use.

2.4 Characterization of synthesized NPs

The UV–vis absorbance spectra (UV and visible light) were measured between 200 and 800 nm to determine the optical properties of the four synthesized trimetallic NPs. To obtain a clear reading for this characterization, the synthesized NP solution was diluted 100 times. Utilizing Fourier transform infrared (FTIR) spectroscopy, the physicochemical properties of synthetic trimetallic NPs were investigated. FTIR analysis was used to determine which functional groups in the *C. roseus* extract were responsible for producing the trimetallic NPs. Dynamic light scattering (DLS) was employed to determine the synthesized trimetallic NPs’ size. The scanning electron microscope (SEM) can be used to gather details regarding the morphology of manufactured NPs.

2.5 Evaluation of the antioxidant activity of synthesized NPs

The trimetallic NPs’ antioxidant activity was assessed using the DPPH test. L-Ascorbic acid was utilized as the typical antioxidant agent along with 0.3 mM DPPH. L-Ascorbic acid was produced for the experiment in different quantities (10, 50, 100, 150, 200, 500, and 1,000 mg·ml$^{-1}$). The same goes for the different concentrations of synthesized NPs that were created (10, 50, 100, 150, 200, 500, and 1,000 mg·ml$^{-1}$). The same procedure was used for L-ascorbic acid: 1 ml of the NP solution was combined with 3 ml of DPPH and incubated in a dark room for 30 min. At 517 nm, the absorbance was measured. The following formula was used to calculate the percentage (%) inhibition:

\[
\frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100 = \% \text{ inhibition}
\]

where $A_{\text{sample}}$ is the absorbance of the test sample and $A_{\text{blank}}$ is the absorbance of the control (which contains ethanol and DPPH).

2.6 Evaluation of the catalytic activity of synthesized NPs

We evaluated the catalytic activity of Cu/Fe/Zn trimetallic NPs by examining the absorbance peaks between 200 and 800 nm using a UV–vis spectrophotometer. It was examined for catalytic activity using an aqueous solution of phenol red (PR) and eosin yellow (EY) in the presence...
of NaBH₄. A mixture of 1 ml of dyes at a concentration of 0.5 mM, 1 ml of NaBH₄, 1 ml of the Cu/Fe/Ag NP solution, and 7 ml of distilled water was used to measure absorbance. Absorbance was tested every 2 h for 6 h at different periods. It was found that organic dyes, such as PR and EY, had reduced throughout the same period. The objective of the study was to examine how time affects dye reduction in the presence of Cu/Fe/Zn NPs by using a UV–vis spectrophotometer to track dye degradation.

3 Results and discussions

3.1 UV–vis studies of synthesized NPs

UV–vis spectroscopy is an analytical technique used to characterize the optical properties of the NPs. UV–vis spectroscopy detects the absorption of light in the UV–vis range (typically 200–800 nm) by the NPs. The absorption spectrum provides information about the electronic transitions occurring within the NPs, which are influenced by their size, shape, and composition. UV–vis spectroscopy analysis was also used to analyze the Cu/Fe/Ag trimetallic NPs. The UV–vis study revealed three different peaks (Figure 2).

A spike in the absorbance band at around wavelength 220 nm indicated the presence of copper oxide. Previous research has reported the absorbance peak of CuO NPs at about 250 nm [23] and 234 nm [24]. Another distinct peak was observed at a wavelength of roughly 270 nm, which may have been an indication of iron oxide NPs. Peaks between 295 and 301 nm were reported in a study that used Avicennia marina flower extract to generate iron oxide NPs [25]. Ag NPs are depicted by a prominent peak that was detected at 370 nm. This is consistent with previous studies that detected Ag peaks at 400 nm [26] and 390 nm [27] regions.

When Ag and CuO interacted to create Ag-doped CuO NPs, a clear peak at around 400 nm was seen in the synthesis of Ag-doped CuO NPs [28]. This UV–vis spectrum further supports the successful formation of the trimetallic NPs.

3.2 FTIR

An effective method for characterizing the functional groups, chemical makeup, and bonding arrangements of the NPs is FTIR spectroscopy analysis [29]. The FTIR spectra of trimetallic NPs and leaf extract from C. roseus are shown in Figure 3. The larger band in the spectrum between 3,000 and 3,500 cm⁻¹, at 3,413 cm⁻¹, is related to the O–H stretching vibration, indicating the presence of alcohols and phenols. Cu/Co/Ni trimetallic NPs containing –OH groups were synthesized with the aid of Origanum vulgare leaf extract, yielding a similar absorbance peak at 3,456 cm⁻¹. Similar to this, in their study on the synthesis of Au–Pt–Ag from Lamii albi flos extract, Dlugaszewska and Dobrucka discovered strong stretching peaks in the FTIR spectra of 3,323 and 3,295 cm⁻¹ corresponding to the OH groups [30]. It appears that the peaks at 571.6, 534.8, and 661.5 cm⁻¹ were caused by the presence of strong alkyl halides, namely C–Cl and C–Br. Zahir et al. studied the biofabrication of Ag and TiO₂ NP using Euphorbia prostrata leaf extract and found that the maximum stretch of S–C was 649 cm⁻¹ [31]. Therefore, it may be concluded that a halogen compound is responsible for the sharp peak at 665 cm⁻¹ in the current investigation. A C=O (carbonyl) stretching vibration is frequently linked to an FTIR absorbance peak at 1,637 cm⁻¹. This kind of functional group is frequently present in substances such as carboxylic acids, ketones, and amides. A peak of 1,644 cm⁻¹, which is near the absorbance peak at 1,637 cm⁻¹, which indicates the existence of a carbonyl group, was demonstrated by Vaseghi et al. in one piece of literature as C=O stretching vibrations in carbonyl groups or the bending of the C–N bond in the amide group of flavonoids and phenolic acids [32]. These results suggest that phenols and flavonoids are likely the components of systems that reduce metal precursors to their corresponding metal ions. In the absence of additional strong ligating agents, polyphenolics can be selectively absorbed into the outermost layer of metal NPs due to π–electron interaction. The π-electrons of the C=O in the flavonoid C ring can move to a metal ion’s free orbital during a redox process, transforming it into a free metal.
The accompanying image displays the FTIR spectra of Cu/Fe/Ag trimetallic NPs separated from C. roseus. The C—O bond seen by the large peak at 1,648 cm\(^{-1}\) is created by stretching vibrations in the carboxyl group or by bending the C–N in the amide group. Additionally, it is possible for amines and amides engaged in the bioreduction of metal precursors for the production of Cu/Fe/Ag trimetallic nanocomposites to be stretched in a bonded NH/C–H/OH manner due to the stretch of the absorption band between 3,000 and 3,500 cm\(^{-1}\).

### 3.3 DLS analysis

DLS is a technique used to measure the size distribution of particles in a suspension or solution. It provides information about the distribution of particle sizes within a sample, typically represented as a histogram or intensity-weighted size distribution curve. The analysis can reveal the presence of monodisperse (uniform size) or polydisperse (varied size) populations of particles. The Cu–Fe–Ag NPs were subjected to DLS analysis and found to have average particle size distribution of roughly 190 nm. In similar studies of silver NP synthesis from L. nodiflora extract, Sudha et al. determined the average particle size, which came out to be 143.7 nm. They concluded that the presence of trace amounts of larger particles created by contamination or agglomeration could be the cause of the larger size and could introduce uncertainty into particle size measurements [2]. Similarly, the particle size was roughly 500 nm while dealing with Coffea arabica to biosynthesize gold NPs. It was determined that the cloud of molecules produced by the plant extract is the cause since it interacts with the NP’s surface to expand its hydrodynamic diameter [33].

The generated Cu–Fe–Ag NPs’ surface charge was also determined using the zeta potential. It was found that the surface charge was \(-13.5\) mV. It is commonly accepted that zeta potential values of more than +30 mV or less than 30 mV result in stable suspensions [34]. Zinc oxide NPs were generated by Supraja et al. using the stem bark extract; however, their zeta potential was limited to 4.8 mV. They concluded that this indicated repelling electrostatic forces acting on zinc NPs [35]. The size and charge of the synthesized NPs are displayed in Figure 4(a) and (b), respectively.
3.4 SEM analysis

SEM is a powerful imaging technique used to observe the surface morphology, topography, and composition of materials at high resolution. The synthesized trimetallic NP exhibited some aggregation and an uneven form, which led to an increase in particle size, according to the SEM research. Particle aggregation was seen in the trimetallic nanocomposites (Figure 5). The fact that the three metals included in the sample under examination showed up in their typical nanoforms could be one reason for this. Since the synthesis takes place in a biological medium, several minuscule agglomerates were also noticed. These agglomerations may impact the functionality of the NPs. The small particulate size provides more surface area and active sites, while agglomeration may limit the active site of the NPs, thus affecting its overall effectiveness.

Figure 4: (a) Zeta potential distribution of Cu/Fe/Ag NPs. (b) The particle size of Cu/Fe/Ag NPs.
4 Antioxidant activity

An antioxidant is a material that stops molecules in cells from oxidizing. Free radicals and reactive oxygen species (ROS) can cause cell or tissue damage as a result of oxidative stress. Oxidative stress is a phenomenon of imbalance between the neutralization of free radicals and their production. To counter these free radicals, an antioxidant is used which can be either organic or synthetic antioxidants [36]. Studies have shown that due to the tiny size and vast surface area, metallic NPs synthesized using biological have potent antioxidant activity because of the presence of natural antioxidants such as vincristine, vinblastine, catechins, curcumin, and allicin. The presence of several different alkaloids in C. roseus, which was used as an extract in the production of the trimetallic NPs, may be related to the antioxidant activity of the NPs. To evaluate the antioxidant activity of the trimetallic Cu/Fe/Ag NPs, a DPPH assay was employed. The DPPH radical is employed in this experiment as a stable free radical that reacts with antioxidants to produce a color shift that can be detected using spectrophotometry. By accepting the hydrogen radical or electron from the donor antioxidant, the purple DPPH reagent is changed into a stable yellow diphenyl picryl hydrazine molecule in this process. Measuring the scavenging activity involves calculating the proportion of DPPH radical inhibition. Ascorbic acid was used as a reference antioxidant in the DPPH experiment to determine the antioxidant capacity of Cu/Fe/Ag NPs (Figure 7). Specifically, at 10, 50, 100, 150, 200, 500, and 1,000 µg·ml$^{-1}$, the activity was seen to be 51.72%, 54.16%, 44.17%, 34.85%, 51.27%, 73.86%, and 69.81%. The outcome implies that, at a lower concentration (10 µg·ml$^{-1}$), the antioxidant qualities of Cu–Ag–Fe NPs are higher (almost twice) than those of ascorbic acid, a recognized antioxidant. Several NPs have had their antioxidant qualities evaluated using the DPPH assay. A study on Ag–CuO and Ag–Fe$_2$O$_3$ bimetallic NPs shows antioxidant activity of 63% and 42% at a concentration of 500 and 1,000 µg·ml$^{-1}$ respectively [9,11].

4.1 Mechanism of antioxidant activity

The mechanism by which metallic NPs exhibit antioxidant activity is their capacity to scavenge ROS and free radicals through different mechanisms (Figure 6). The primary
Antioxidant mechanism involves donating electrons or hydrogen atoms to free radicals, effectively neutralizing them and preventing further damage to cellular structures. This scavenging action terminates the chain reaction of oxidative stress. Another mechanism involves activating the body's endogenous antioxidant defense system by upregulating the expression and activity of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase or chelating metal ions, particularly transition metals such as iron and copper, which catalyze the formation of ROS through Fenton and Haber–Weiss reactions. The trimetallic NPs' antioxidant activity may also be enhanced by the several metal atoms' synergistic effects, which enhance their stability and reactivity. Further research is necessary to fully comprehend the mechanism underlying the antioxidant effect of trimetallic NPs.

Figure 7: Antioxidant activity of Cu/Fe/Ag NPs.

Figure 8: Mechanism of catalytic degradation.
Figure 9: (a) Catalytic degradation of EY using Cu/Fe/Ag. (b) Catalytic degradation of PR using Cu/Fe/Ag.
5 Catalytic activity

Dye degradation is the process by which dye molecules are broken down into less toxic forms. Dye degradation is crucial because, if improperly treated before being dumped into water bodies, colors can seriously endanger both human health and the environment. There is growing future concern about the impact of dye use, thus prompting efforts to develop an eco-friendly and sustainable method for dye degradation. When dye molecules are broken down into less toxic compounds by an adsorbent, this process is known as the catalytic degradation of dyes. Scientists have employed NPs as nano-catalysts for this purpose. NPs are known for their high surface area, which enables quick electron transfer in the presence of NaBH₄, leading to reduction and degradation of dyes. The degradation process can occur through various mechanisms, including adsorption, reduction, oxidation, and photodegradation. In the case of adsorption, it provides active sites to attach to, which can lead to further degradation. Reduction is the process by which electrons from the adsorbent are transferred to the dye molecules, changing them into less toxic byproducts. This process is frequently seen in the azo dye breakdown process. By moving oxygen atoms from the adsorbent to the dye molecules, a process known as oxidation, the dye molecules are changed into less toxic byproducts. Reactive dye degradation is a frequent mechanism observed in several chemical reactions. In this work, trimetallic NPs were used as nanaoadsorbents in the presence of NaBH₄ as a reducing agent to efficiently degrade dyes.

Due to the toxicity and stability of dye molecules such as EY and PR, it has become essential to remove these dyes from water sources for the protection of the environment and the benefit of human health (Figure 8). EY is a watersoluble anionic dye that falls into the category of fluorescein dyes. Its byproducts are highly toxic and carcinogenic to the environment [37]. Similarly, PR is a pH indicator commonly used in cell culture medium, microbiology, and other diagnostic tests. PR contains two phenolic groups (–OH) and a sulfonate group (–SO₃H) that contribute to its acidic properties. PR is typically supplied as a sodium salt, which makes it more water-soluble and stable in solution.

Figure 9(a) and (b), respectively, displays the absorption spectra of the degradation of PR and EY by NaBH₄ in the presence of Cu/Fe/Ag as catalysts. With a peak absorption wavelength of about 520–530 nm, xanthene dye EY is known to absorb light in the visible region of the electromagnetic spectrum. It is clear that during 6 h, EY deteriorates by 48.6%. Meanwhile, PR was 76% eliminated 6 h after Cu/Fe/Ag was added to the reaction mixture. The high surface-to-volume ratio of the NPs increases the reactivity of the ration mixture. The higher reactivity of the NPs can be attributed to their vast and distinctive surface area. Moreover, the presence of a reducing agent layer on the outside of trimetallic NPs can promote efficient dye molecule adsorption onto them 25. The transport of electrons from the donor to the acceptor is facilitated by metal NPs. The large surface area of the NPs serves as a substrate for the electron transfer process. It is expected that once dye molecules adsorb onto the active sites of NPs, there will be an electron transfer from the NPs to the dye molecules, changing the dye molecules into less toxic products. In several recent studies, metallic NPs have been used to help in break down of dangerous dyes. For example, the degradation efficiency of methyl orange hit over 100% after just 1 minute of incubation using Fe/Cu/Ag NPs. Furthermore, because of the complementary effects of their metallic counterparts, trimetallic NPs exhibit greater catalytic activity than mono- or bimetallic NPs.

6 Conclusion

In conclusion, this research expands our understanding of the synthesis of Cu/Fe/Ag trimetallic NPs using the C. roseus leaf extract, which serves as both the reducing and stabilizing agent. The synthesized trimetallic NPs were found to be 190 nm according to DLS analysis. The synthesized trimetallic NPs exhibited their maximum antioxidant activity at 500 µg·ml⁻¹, or 73.86% which highlights its potential to be used as food packaging material. It was also discovered that the highest catalytic degradation of PR and EY was 76% and 48.6%, respectively. Overall, the green synthesis approach aligns with sustainable practices, promoting the development of eco-friendly nanotechnologies, and demonstrates that dye contaminants in water samples can be remedied using synthesized trimetallic NPs. This shows promising results while further study studies are required for application in different fields.

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