

Biosynthesis, Characterisation, and Antioxidant Activity of Silver Nanoparticles using *Schinus molle* L.

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Abstract

Schinus molle L. has been used as a folk medicine for many years. The aim of this study is to synthesize the silver nanoparticles, to identify them by spectroscopic techniques and to evaluate the antioxidant effects. In this study, *Schinus molle* was extracted with hexane, dichloromethane, ethyl acetate and methanol successively. After removal of the solvent from each extract by reduced pressure, the crude extracts were yielded. Methanol extract was used for synthesis of silver nanoparticles (s-AgNPs). The methanol extract dissolved in distilled water was treated with silver nitrate solution to produce the s-AgNPs. The extensive spectroscopic study enabled the identification of synthesized s-AgNPs. UV-Vis spectroscopic analysis presented the maximum absorption of s-AgNPs at 486 nm. The average particle size of s-AgNPs was measured as 77.6 nm by Scanning electron microscope (SEM). X-ray diffraction (XRD) pattern revealed that the nanoparticles were face-centered cubic structure. The slight differences between extract and AgNPs in FTIR spectrum proved the formation of s-AgNPs. In that, some functional group such as hydroxyl in the extract was oxidized while reduction of silver ions. Elemental analysis displayed the silver percentage to be 75.79 %w/w. Antioxidant activity study including DPPH, ABTS⁺⁺ and FRAP assays displayed the considerable effect of s-AgNPs. This is the first report to present the synthesis of silver nanoparticles from *Schinus molle* L. methanol extract and to evaluate the antioxidant activity.

Keywords: *Schinus molle*, Nanoparticles, Spectroscopy, Antioxidant activity

Introduction

Nanotechnology is one of the most interesting subjects used to produce materials with interatomic structural properties. Due to the morphology and size of nanoparticles, they exhibit distinctive features [1,2]. The biosynthesis of nanoparticles with the desired shape, size and crystal structure has been one of the main purposes of chemistry due to their effective application such as catalysis, biomedical, biolabeling, biosensor, solar cells as well as anticancer, antioxidant, antifungal agents [3-6].

Physio-chemical techniques have been employed for synthesis of nanoparticles. Due to the drastic reaction condition and toxic residue of corresponding techniques, green synthetic approaches are gaining considerable attention on nanostructure. Mostly, green synthesis including regulation, clean up, control, improvement processes can strengthen their eco-friendliness. Some basic principles of biosynthesis can thus be defined by various factors such as pollution reduction, use of non-toxic solvent, prevention of waste and renewable raw material stock. Biosynthesis is crucial to prevent the formation of harmful by-products with an environmentally friendly and sustainable approach. Plant extracts, bacteria and algae have been used for biosynthesis of metal and metal oxide nanoparticles. Among them, using the plant is a rapid, cost-effective, facile and simple process to synthesize nanoparticles at a large level [7,8]. Moreover, green nanomaterials possess a great application in the pharmaceutical market such as pharmaceutical raw materials, drug delivery, functional nanodevices.

Aromatic and medicinal plants are the main source of primary healthcare in many nations due to their bioactive constituents [9-12]. The improvement of spectroscopy and chromatography led to the isolation of bioactive compounds from the plants. Therefore, the plants especially aromatic and medicinal plants became the focus of science [13-16]. Due to the side effect of synthetic chemicals, the trend in natural products in health and drug development has increased recently [17].

The black currant pomace extract was used for the synthesis of AgNPs. LC-MS study presented the presence of 16 compounds with the caffeic acid and chlorogenic acid as the major compounds. The AgNPs showed the efficient antioxidant activity [18]. In addition, AgNPs were synthesized using the black currant and apricot pomace aqueous extract and LC-MS study revealed the compounds responsible for reducing, stabilization and antioxidant activity [19].

Schinus molle L. is a pepper tree belonging to the Anacardiaceae family. It is native to South America but distributes to tropical and subtropical areas. The essential oils from leaves and fruits have been used in perfumery and culinary. In addition, it is commonly employed as an ornamental plant in Europe. The essential oil and extract of this plant have been reported to have antimicrobial, antifungal, anti-inflammatory, antispasmodic, antipyretic, antitumor and cicatricial properties [20-22]. The main essential oils compounds were α -phellandrene, limonene, α -eudesmol, elemol [23]. *S. molle* has been used in traditional medicine as antidepressant, stimulant, tonic, antihemorrhagic, antiseptic, digestive stimulant, menstrual stimulant, diuretic, cardiotoxic [24].

Free radicals, highly reactive oxygen species, are important components of many reactions in eukaryotic cells. Excess free radicals can be produced under stress and bad living habits cause oxidative damage to lipids, proteins and nucleic acid. This damage causes some diseases. Therefore, many antioxidant-based drug designs have been used for the treatment of some diseases such as cancer, Alzheimer's disease, and diabetes. Recently, there has been rapid interest in finding the naturally occurring antioxidant for use in food, cosmetics and medical materials to replace synthetic antioxidants that are restricted due to their carcinogenicity [25]. As a result, plant-mediated silver nanoparticles could be an outstanding antioxidant agent for food, cosmetic and medicinal materials.

The bioactive compounds of the plant play a significant role in the reduction of silver ions to synthesize nanoparticles and contributing to their stability [26]. Herein, silver nanoparticles were synthesized with low-cost, convenient, eco-friendly using methanol extract of *Schinus molle*.

Materials and methods

Chemicals

The chemicals and solvents with analytical grade for synthesis of silver nanoparticles and antioxidant assays were supplied from Merck KGaA, Darmstadt, Germany.

Plant material

Schinus molle L. was collected from Biskra, Algeria on 12 July 2019. Botanical identification was carried out by Prof. Dr. Ozgur Eminagaoglu, Artvin Coruh University, Faculty of Forestry. A voucher specimen was deposited at the Faculty of Forestry, Artvin Coruh University (ARTH 16704).

Extraction procedure

Air dried plant material (200 g) was extracted with hexane for 3 days. After filtration, the solvent was removed by reduced pressure to yield the crude hexane extract. The solid material was re-extracted with dichloromethane, ethyl acetate and methanol sequentially to yield the crude extracts. The methanol extract was used for synthesis of s-AgNPs.

Synthesis of silver nanoparticles

The methanol extract of *Schinus molle* (1.0 g) was dissolved in distilled water (300 mL). The silver nitrate solution (0.024 M, 300 mL) was added to the extract solution slowly. The final reaction mixture was mixed at 55 °C for 2 h. The colour change from yellow to brown showed the establishment of s-AgNPs. The mixture was cooled to room temperature then, applied to centrifugation at 15,000 rpm for 10 min, washed with distilled water. After drying by lyophilisation, s-AgNPs were yielded [27].

Identification of silver nanoparticles

The synthesized s-AgNPs were characterized by fully spectroscopic study. Hitachi U-2900 spectrophotometer was used for UV-Vis measurement. The functional groups of secondary metabolites in the extract and capped nanoparticles were revealed by FTIR (FT/IR 4,700 spectrometer) spectroscopic

analysis. The diffraction pattern for XRD analysis was recorded by Malvern Panalytical diffractometer. The particle size was calculated by Dynamic light scattering (DLS) on a Delsa Nano C instrument. Quanta Feg450 was used for SEM analysis and elemental analysis was carried out by EDS (Energy dispersive X-ray spectroscopy) combined with SEM microscopy.

DPPH[•] free radical scavenging assay

1,1-diphenyl-2-picrylhydrazyl free radical, DPPH[•] scavenging effect of *S. molle* mediated s-AgNPs was executed [28]. s-AgNPs at various concentration was treated with DPPH[•] radical (0.26 mM) for 25 min at room temperature. BHT, BHA and Trolox were utilized as standards. The results are presented as IC₅₀ values indicating the concentration of silver nanoparticles that scavenge 50 % of the DPPH[•] free radical.

ABTS^{•+} radical cation activity

ABTS (2.0 mM) solution was treated with the (K₂S₂O₈) (2.45 mM) to yield the ABTS^{•+} which was kept in dark for 5 h at rt. ABTS^{•+} solution was treated with sodium phosphate buffer. Later, treatment of nanoparticles at several concentrations with ABTS^{•+} was executed. Hence, the absorbance was measured at 734 nm [29].

Reducing power

The s-AgNPs (0.1 mL, 40 - 150 µg/mL) were treated with potassium ferricyanide (1.25 mL, 1 %) and phosphate buffer afterward, incubation was carried out at 50 °C for 20 min. FeCl₃ (0.25 mL, 0.1 %) and trichloroacetic acid were added to the reaction flask then it was vortexed for 3 min. The absorbance was measured at 700 nm in a spectrophotometer [30].

Statistical analysis

Statistical analysis was executed by Graph Pad Prism software (version 8.00) with 1-way ANOVA. The results were reported as mean values ± SDs of 3 independent assays ($p < 0.05$).

Results and discussion

Synthesis of silver nanoparticles

S. molle was extracted with hexane, dichloromethane, ethyl acetate and methanol sequentially to yield the crude extracts (Yield of each extract was 5 - 8 %). In general synthesis of AgNPs from plants, the plant is heated in distilled water then it is treated with silver nitrate. But many apolar molecules such as fatty acids can affect the reaction. In this extraction method, fatty acid and apolar molecules dissolve in hexane and dichloromethane extract. Hence, polar molecules such as flavonoids, which have functional groups are used for reduction of silver ions and stabilisation. In this work, the methanol extract was used for synthesis of s-AgNPs. Initially, methanol extract was dissolved in distilled water and treated with AgNO₃ solution to reduce the Ag⁺ to Ag⁰. The formation of *S. molle* mediated s-AgNPs were revealed by the colour change from yellow to dark brown (**Figure 1**).

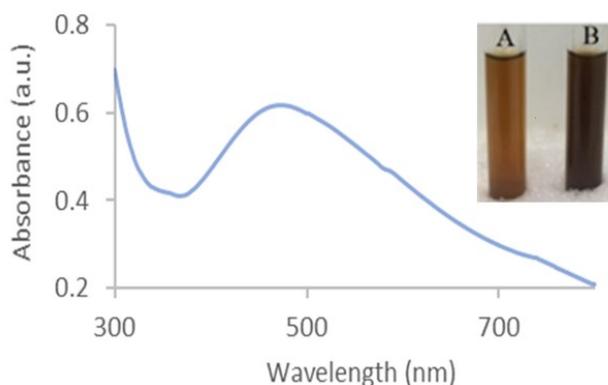


Figure 1 Aqueous solutions of (A) plant extract, (B) AgNPs, and (C) UV-Vis absorption spectrum of the biosynthesized s-AgNPs at 473 nm.

The plant extracts include the secondary metabolites such as flavonoids, terpenoids, steroids, alkaloids, phenolic acids, etc. These compounds act as reducing agent for the silver ions. A flavonoid was selected for presenting the plausible mechanism. In the reaction mechanism (**Figure 2**), silver ions were reduced by the functional groups of extract bioactive compounds of which oxidized. The silver nanoparticles were formed in the stage of ion reduction, clustering and growth of the nanoparticles [31]. The reduction and oxidation reaction of aqueous AgNO_3 solution mediated by plant solution led to the formation silver nanostructure.

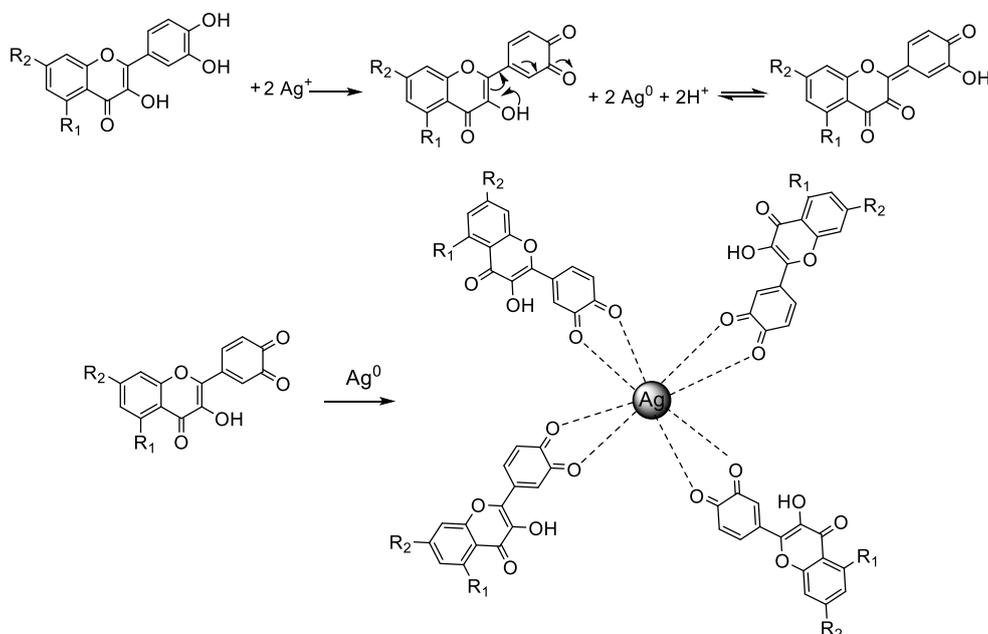


Figure 2 Plausible reaction mechanism of silver nanoparticles synthesis (s-AgNPs).

UV-Vis spectral analysis

The UV-vis spectroscopic technique is commonly used for the elucidation of nanoparticles. The various metal nanoparticles in the size range from 2 to 100 nm were identified at 300 - 800 nm wavelengths. The absorption in the wavelength ranging from 400 to 450 nm reveals the establishment of silver nanoparticles [32]. In this study, the maximum absorption at 473 nm at UV-Vis spectrum revealed the silver nanoparticles formation (**Figure 1**).

Fourier-transform infrared spectroscopy (FTIR) analysis

FTIR spectrum presented the functional groups responsible for the silver ion reduction and stabilization of s-AgNPs. The absorption differences in the FTIR spectrum of s-AgNPs and *S. molle* extract confirmed the formation of s-AgNPs. These differences are caused by the oxidation of functional groups such as hydroxyl while reduction of silver ions. The characteristic vibration signals of extract and AgNPs for hydroxyl groups appeared at $3,254$ and $3,270 \text{ cm}^{-1}$, respectively. The signal observed for extract and s-AgNPs at $2,925$ and $2,921 \text{ cm}^{-1}$ could be due to the CH stretching signal. The signals observed at $1,606$ and $1,626 \text{ cm}^{-1}$ for extract and s-AgNPs belonged to the C=C stretching and carbonyl, respectively. The N-O stretching signal in the extract appeared at $1,518 \text{ cm}^{-1}$ and C-H bending of methyl group signal in s-AgNPs was observed at $1,454 \text{ cm}^{-1}$. The stretching vibrations in the extract at $1,442$ and $1,361 \text{ cm}^{-1}$ represent O-H bending. The signals at $1,375$ and $1,170 \text{ cm}^{-1}$ in s-AgNPs could be attributed to the C-H bending of aldehyde and C-O stretching of alcohol, respectively. The peaks that appeared at $1,240$ and $1,202 \text{ cm}^{-1}$ in extract might be due to the C-N stretching and C-O stretching, respectively. Besides, s-AgNPs signals at $1,063$ could belong to C-O stretching. Hence, Ag^+ silver ions could be reduced to Ag^0 by plant metabolites such as proteins and flavonoids. Moreover, s-AgNPs were capped and stabilized by functional groups (**Figure 3**).

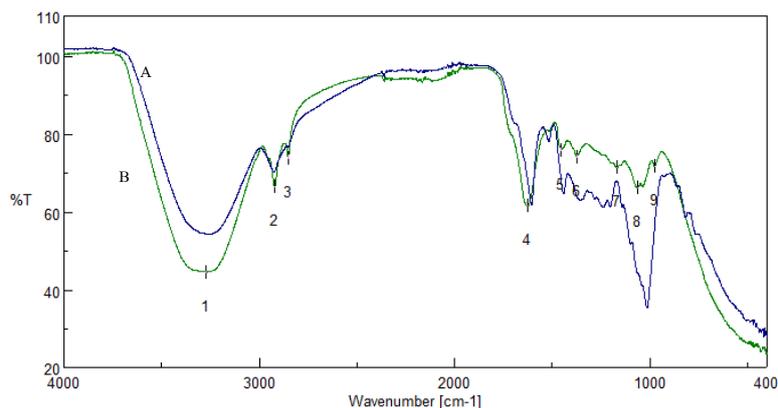


Figure 3 (A) IR spectrum of extract and (B) silver nanoparticles. The corresponding wave number to number for extract, 1: 3,254, 2: 2,925, 3: 1,606, 4: 1,518, 5: 1,442, 6: 1,361, 7: 1,240, 8: 1,202, 9: 1,014. s-AgNPs, 1: 3,270, 2: 2,921, 3: 2,851, 4: 1,626, 5: 1,454, 6: 1,375, 7: 1,170, 8: 1,068, 9: 974.

X-ray diffraction (XRD) analysis

The crystal structure of s-AgNPs were elucidated by X-ray diffraction analysis (**Figure 4**). The XRD pattern of synthesised s-AgNPs revealed the distinctive peaks indexed to the crystalline planes of face centred cubic silver. The intense peaks at 2θ of 38.1, 44.3, 64.4 and 77.4 ° correspond to (111), (200), (220) and (311) planes, respectively which confirmed by Joint Committee on Power Diffraction Standards (JCPDS No. 04-0783) report [33]. The extra peaks were due to the impurities caused from silver nitrate.

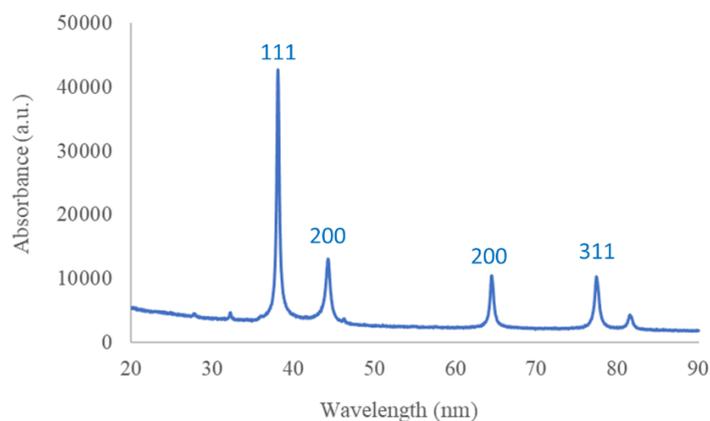


Figure 4 XRD pattern of s-AgNPs.

Scanning electron microscope (SEM) and energy-dispersive x-ray spectroscopy (EDX) analysis

The scanning electron microscope (SEM) presented the surface morphology and size of s-AgNPs (**Figure 5**). The energy dispersive analysis of x-rays (EDX) showed the presence of AgNPs. Furthermore, remarkable intense peak of s-AgNPs in EDX spectrum at around 3 and 3.3 keV confirmed the synthesis of AgNPs (**Figure 5**). SEM analysis of synthesised s-AgNPs were spherical shaped and measured as an average size of 77.6 nm. Moreover, elemental analysis results indicated that the silver percentage to be 75.79 %.

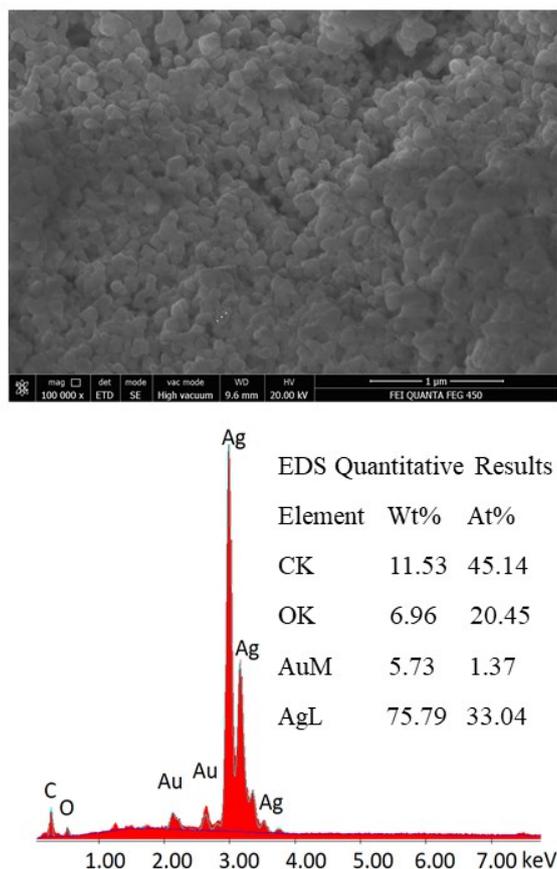


Figure 5 SEM image, EDX spectrum and elemental analysis of s-AgNPs.

Antioxidant activity

Antioxidant activity of s-AgNPs was carried out using DPPH, ABTS and FRAP assays. The extract revealed excellent DPPH scavenging effect (IC_{50} , $\mu\text{g/mL}$, 8.44) comparing to the standard, BHT (IC_{50} : 10.96 $\mu\text{g/mL}$). s-AgNPs showed the moderate activity. In ABTS⁺ radical cation activity, s-AgNPs displayed the excellent activity (IC_{50} : 3.56 $\mu\text{g/mL}$) in comparison to the standards, BHT, BHA and Trolox. In addition, the extract activity (IC_{50} : 5.47 $\mu\text{g/mL}$) was significantly higher than that of the standard BHT (IC_{50} : 7.28 $\mu\text{g/mL}$). s-AgNPs and extract displayed the moderate reducing power activity in comparison with the standard. As a consequence, the s-AgNPs and extract displayed the high antioxidant activity (**Figure 6**). Hence, s-AgNPs and extract have a potential to be used as an antioxidant agent in food industry. Medicinal plants mostly display the antioxidant effects due to their bioactive compounds such as phenolic compounds. Scientific studies have revealed that the consumption of antioxidant-rich foods is inversely related to human diseases [34]. Plants that have been used for medicinal purposes for thousands of years are the basis of traditional medicine. Effective techniques have been developed to obtain natural antioxidants from plants and use them in the food, and pharmaceutical industries [35]. Medicinal plants and their secondary metabolites have various biological effects such as anti-inflammatory, anti-carcinogenic and antiatherosclerotic effects because of their antioxidant activities [36]. Many plant extracts and compounds isolated from plants were reported to display antioxidant activity [37,38]. Moreover, green synthesized silver nanoparticles revealed excellent antioxidant activity [39,40].

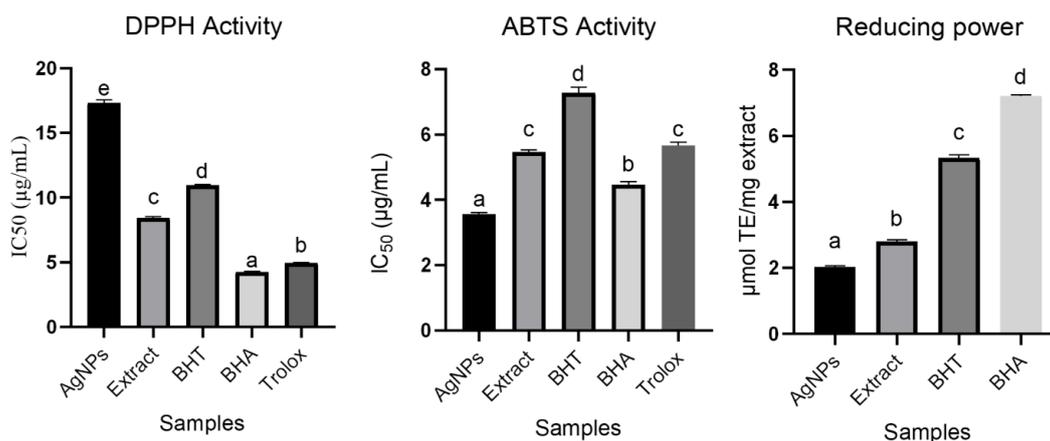


Figure 6 Antioxidant activity of AgNPs and extract. The results were reported as mean values \pm SDs of 3 independent assays ($p < 0.05$). Values followed by the same letter are not significantly different.

Conclusions

The biosynthesis of nanoparticles has been advanced and has been an attractive field of science research in the last decades. Therefore, the use of green chemistry knowledge and green approach for the synthesis of nanoparticles is increasing steadily to achieve an eco-friendly procedure. In this study, silver nanoparticles were synthesized as a low-cost, easy and eco-friendly process. The spectroscopic studies defined the nanoparticles as face-centered cubic unit structures. The bioactive compounds in the extract acted as reducing, stabilizing and capping agents. Due to the increase interest in natural products in pharmacy, *Schinus molle* extract and *S. molle* mediated silver nanoparticles could be an antioxidant agent to be used in food industry.

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