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Separation, Identification, and Antidiabetic Activity of Catechin Isolated from *Arbutus unedo* L. Plant Roots

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Received: 10 March 2018; Accepted: 9 April 2018; Published: 12 April 2018



Abstract: Phytopharmaceuticals play an essential role in medicine, since the need to investigate highly effective and safe drugs for the treatment of diabetes mellitus disease remains a significant challenge for modern medicine. *Arbutus unedo* L. root has various therapeutic properties, and has been used widely in the traditional medicine as an antidiabetic agent. The current study aimed to isolate the pharmacologically active compound from *A. unedo* roots using accelerated solvent extraction technology, to determine its chemical structure using different instrumental analytical methods, and also to evaluate the α -glucosidase inhibitory activity. The roots of *A. unedo* were exhaustively extracted by high-pressure static extraction using the Zippertex[®] technology (Dionex-ASE, Paris, France), and the extract was mixed with XAD-16 resin to reach quantifiable amounts of active compounds which were identified by high-pressure liquid chromatography (HPLC), ¹H NMR (300 MHz), and ¹³C NMR. The antidiabetic activity of the isolated compound was evaluated using the α -glucosidase inhibitory assay. The active compound was isolated, and its structure was identified as catechin using instrumental analysis. The results revealed that the isolated compound has potential α -glucosidase inhibitory activity with an IC₅₀ value of 87.55 ± 2.23 μ g/mL greater than acarbose. This was used as a positive control, which has an IC₅₀ value of 199.53 ± 1.12 μ g/mL. According to the results achieved, the roots of *A. unedo* were considered the best source of catechin and the Zippertex[®] technology method of extraction is the best method for isolation of this therapeutic active compound. In addition, the α -glucosidase inhibitory activity results confirmed the traditional use of *A. unedo* roots as an antidiabetic agent. Future clinical trials and investigations of antidiabetic and other pharmacological effects such as anticancer are required.

Keywords: *Arbutus unedo* L.; α -glucosidase; catechin; HPLC; NMR

1. Introduction

Diabetes mellitus is a disorder of carbohydrate metabolism characterized by the impaired ability of the body to produce or respond to insulin, and thereby maintain proper levels of glucose in the blood. This produces several devastating effects and complications, including neuropathy, nephropathy, retinopathy, hyperthyroidism, hypertension, arteriosclerosis, and many other serious diseases [1–3]. In fact, other complications are associated with the used antidiabetic drugs, as regular administration can lead to several adverse effects [4]. Accordingly, the achievement of sufficient control of hyperglycemia is difficult to reach with commercially available antidiabetic medications, thereby resulting in various and serious complications [5]. In fact, the investigation on medicinal plants usually started with extraction procedures which play a crucial role in the extraction outcomes, e.g., yields percentages and the quality of the produced phytochemicals. Nowadays, a wide range of technologies with different methods of extraction is available. Zippertex technology is one of the most efficient methods of extraction. It is a high-pressure static extractor which is considered the most efficient and convenient solid/liquid extraction device. It combines extraction and filtration steps, offering limpid highly concentrated extracts, ready for chemical and biological investigations. The combination of static pressure and heating favor the access of the solvent into the heart of the solid matter, and increases the solubilization of the target compounds. Zippertex offers the maximum qualitative and quantitative recovery, with the minimum required operations, solvents, handling, and time [6,7].

Medicinal plants offer a great opportunity to discover new natural therapeutic molecules. Some of these molecules may have beneficial effects on glucose homeostasis in diabetic patients without causing any undesirable effects currently observed in modern antidiabetic agents [5,8].

Arbutus unedo L. (Ericaceae family) is commonly known as wild strawberry, which is a perennial small tree native to the Mediterranean basin countries. It constitutes an important contribution to the nutritional culture and to the health promotion of Moroccan community [9,10].

However, in traditional medicine of many Mediterranean countries, *A. unedo* plant has been used widely with the employment of decoctions and infusions of all plant parts: fruits, leaves, barks, and roots [11,12]. For instance, the fruits are well known in folk medicine as an antiseptic, laxative, and diuretic [13,14], while the leaves are used as an astringent, urinary antiseptic, diuretic, antidiarrhea, and depurative. Moreover, recently, the leaves have been used to treat inflammatory diseases, hypertension, and diabetes [11].

Furthermore, *A. unedo* roots have been used traditionally to treat various gastrointestinal, urological, dermatologic, and cardiovascular diseases, as well as a diuretic, anti-inflammatory, and antidiabetic agent [15–18].

In fact, the *A. unedo* extract allowed us to identify several familial compounds, such as terpenoids, free quinine, and anthraquinone, which were the subject of our previous work and others, such as polyphenols, flavonoids, and tannins, which were quantified in the plant roots aqueous extract, thus, its antioxidant activity was also evaluated [10].

The current study aimed to isolate the therapeutic active molecule of *A. unedo* roots using accelerated solvent extraction Zippertex technology with XAD-16 resin, characterize its structure using different spectra methods and to evaluate its α -glucosidase inhibitory activity.

2. Results

The results of the present investigation showed that the extraction of *A. unedo* roots utilizing the Dionex-ASE (accelerated solvent extraction) prototype Zippertex produced a high percent of aqueous extract yield with 23.7%. The HPLC chromatogram of *A. unedo* roots aqueous extract showed that this extract contained several phytochemical compounds, as shown in Figure 1A. This makes it difficult to identify the pharmacologically active principle, which encouraged us to look for another experimental method of separation, from which we can isolate a single therapeutic molecule. Satisfactory results were obtained using the XAD-16 resin. After adsorption on XAD-16 resin, the expected compound

from the extract was separated using semi-preparative HPLC technique, and its chemical structure was identified as shown in Figure 1B.

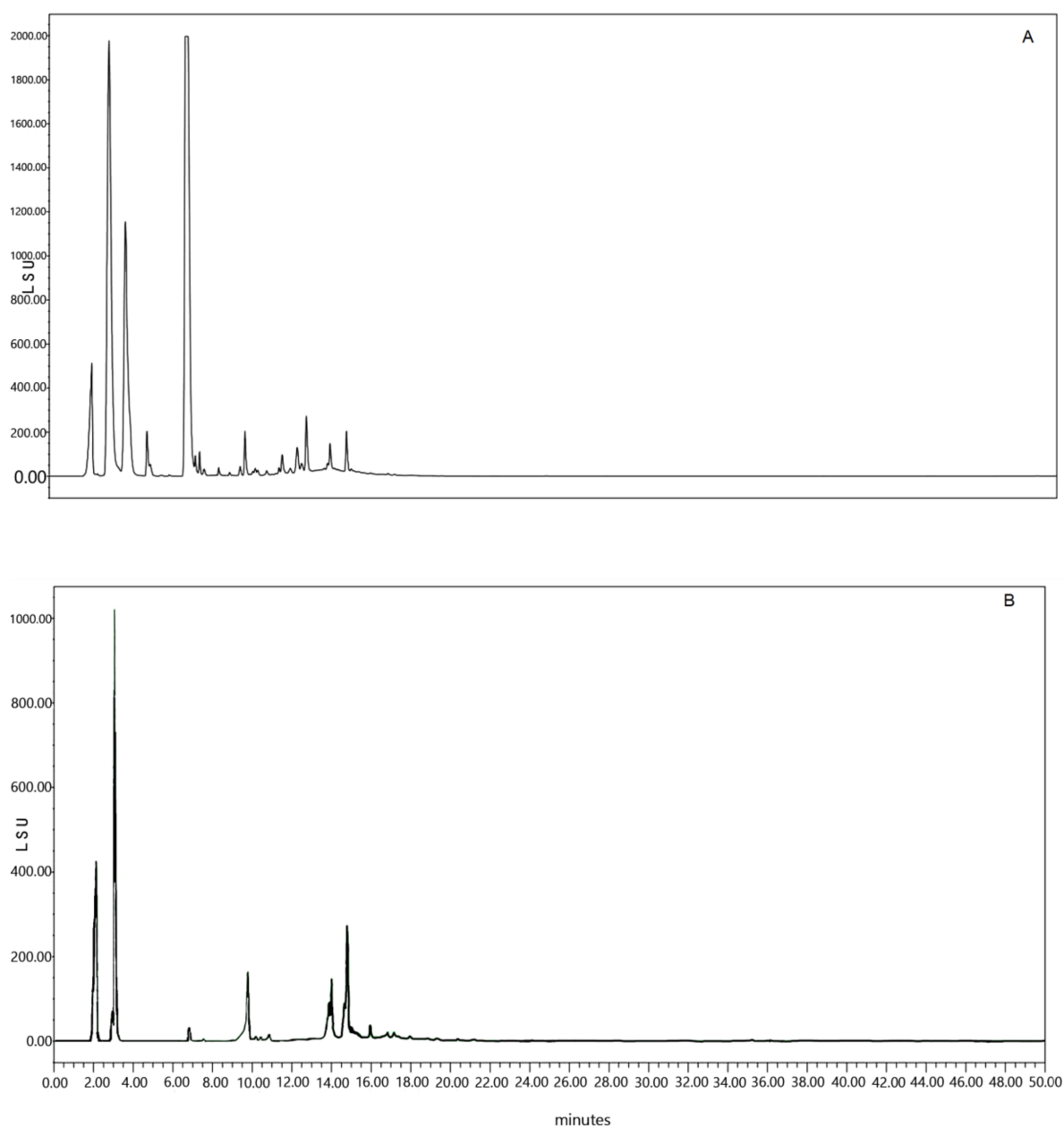


Figure 1. (A) HPLC analysis of *Arbutus unedo* roots aqueous extract obtained by Zippertex apparatus. (B) The aqueous extract of *A. unedo* roots adsorbed by the amberlite XAD-16.

The isolated compound showed interesting α -glucosidase inhibitory activity, results as shown in Table 1, in comparison with commercially used α -glucosidase inhibitory drug acarbose. These results encouraged us to identify and characterize the structure of this compound (Figure 2) using high-resolution mass spectrometry and NMR (^1H and ^{13}C) analyses. The negative-ion HRESIMS analysis gave the molecular formula $\text{C}_{15}\text{H}_{14}\text{O}_6$ on the basis of m/z 289.10 $[\text{M} - \text{H}]^-$ (calculated for $\text{C}_{15}\text{H}_{13}\text{O}_6$) containing nine unsaturation.

The ^1H and ^{13}C NMR spectra show the following signals:

^1H NMR (MeOD, 300 MHz): 2.46–2.54 (m, 1H, $\text{CH}_{2(2)}$); 2.81–2.88 (m, 1H, $\text{CH}_{2(2)}$); 3.29 (s, 1H, $\text{OH}_{(a)}$); 3.97 (dd, 1H, $\text{CH-OH}_{(1)}$, $J = 7.5$ Hz, $J = 2.4$ Hz); 4.57 (d, 1H, $\text{O-CH}_{(9)}$, $J = 7.5$ Hz); 4.85 (s, 4H, $4\text{OH}_{(b,c,d,e)}$); 5.86 (d, 1H, $\text{CH}_{(5)}$, $J = 2.4$ Hz); 5.93 (d, 1H, $\text{CH}_{(7)}$, $J = 2.1$ Hz); 6.69–6.78 (m, 2H, $2\text{CH}_{(14,15)}$); 6.84 (d, 1H, $\text{CH}_{(11)}$, $J = 1.8$ Hz).

^{13}C NMR (MeOD, 75 MHz): 27.09 ($\text{CH}_{2(2)}$); 67.40 ($\text{CH}_{(1)}$); 81.43 ($\text{CH}_{(9)}$); 94.17 ($\text{CH}_{(7)}$); 94.96 ($\text{CH}_{(5)}$); 99.49 ($\text{C}_{(3)}$); 113.89 ($\text{CH}_{(11)}$); 114.76 ($\text{CH}_{(14)}$); 118.69 ($\text{CH}_{(15)}$); 130.82 ($\text{C}_{(10)}$); 144.81 ($\text{C}_{(13)}$); 144.84 ($\text{C}_{(12)}$); 155.51 ($\text{C}_{(8)}$); 156.15 ($\text{C}_{(4)}$); 156.39 ($\text{C}_{(6)}$).

The ^1H and ^{13}C NMR spectra are shown in the Supplementary Material (Figures S1–S6).

The α -glucosidase inhibitory assay results showed that catechin inhibited α -glucosidase enzyme more than two folds of acarbose with IC_{50} value 87.55 ± 2.23 $\mu\text{g}/\text{mL}$ against 199.53 ± 1.12 $\mu\text{g}/\text{mL}$ of acarbose, as represented in Table 1.

Table 1. The α -glucosidase inhibitory potency of catechin.

Compounds	α -Glucosidase Inhibitory Activity IC_{50} ($\mu\text{g}/\text{mL}$), $\pm\text{SD}$
Catechin	87.55 ± 2.23
Acarbose	199.53 ± 1.12

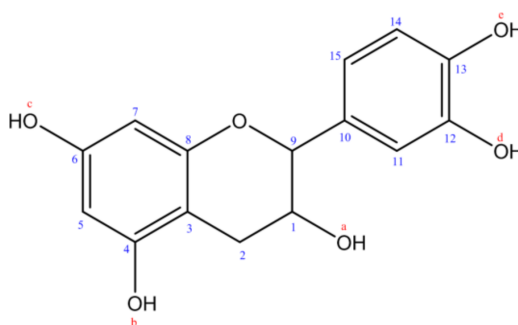


Figure 2. Catechin chemical structure isolated from *A. unedo* roots using XAD-16 resin.

3. Discussion

Due to problems associated with high processing temperatures and long processing times in conventional extraction methods, which can degrade or undergo undesirable oxidation processes, there is an essential need to promote development and application of alternative extraction techniques for phenolic compounds. Accelerated solvent extraction is a promising eco-friendly alternative providing exceptional separation and protection from degradation of unstable polyphenols. The current investigation revealed that extraction method of *A. unedo* roots utilizing of the Dionex-ASE (accelerated solvent extraction) prototype Zippertex produced a high yield of aqueous extract. Several conducted studies showed that the modern techniques, which have been replacing conventional ones, include accelerated solvent extraction, microwave-assisted extraction, supercritical fluid extraction, pressurized liquid extraction, and ultrasound-assisted extraction. These alternative techniques increased the extraction yields of polyphenols, reduced considerably the use of solvents, and accelerated the extraction process [19–21].

Flavonoid is a class of phytogetic polyphenolic compounds, including in many kinds of human diet, and has various physicochemical characters and chemical structures. The high-resolution mass spectrometry and NMR results revealed the presence of catechin in the roots of *A. unedo* extract, and these results are similar to those which were conducted by Junior et al. [22].

In fact, *A. unedo* fruits are considered as an alternative source of flavan-3-ols, in particular, catechin and its derivatives [2]. However, the inhibitory effect of catechin against α -glucosidase enzyme

was evaluated in several previously conducted studies which initially demonstrated that catechin preferentially inhibited maltase rather than sucrase in an immobilized α -glucosidase inhibitory assay system [23–26]. This suggests that the α -glucosidase inhibition induced by catechin is closely associated with the presence of a free hydroxyl group at the 3-position of this molecule [23]. Catechin is a very common and widely diffused metabolite in the plant kingdom. The traditional use of *A. unedo* as antidiabetic agent may be associated with other phytoconstituents, because many plants contain catechin. Therefore, the elevated biological property of this plant is due to the whole phytocomplex, and not only to just one molecule [27,28].

In addition, several conducted studies pointed out the interest of using catechin for many of health benefits, such as anticancer, antifungal, antioxidant, and antidiabetic purposes [29,30].

However, in a study established by Albuquerque et al. [21], they attempted the optimization of extraction of this molecule using different extraction methods, such as maceration and microwave techniques, and were capable of yielding 1.38 ± 0.1 and 1.70 ± 0.3 mg/g DW of catechin, respectively using the optimal extraction conditions. These extraction methods were found to be the most effective methods. The catechin yield from *A. unedo* root in the present study was of 95 mg of the plant dry weight. This high yield, which was obtained by using the Dionex-ASE (accelerated solvent extraction) prototype, Zippertex, in addition to the XAD-16 resin, which was used as an adequate option for an efficient cleanup step for purification and isolation of pure catechin compound from the plant root extract [31]. This yield was considered high in comparison with previously conducted extraction methods [21]. Moreover, the roots of *A. unedo* was considered the best source for isolation of the pharmacologically active molecule catechin in comparison with previously conducted isolation procedures of catechin from different sources [21]. However, many conducted research studies have demonstrated that the Zippertex method is friendly to the environment, with low cost and a low requirement for solvents, which decreased the time of extraction procedure and combined extraction/filtration steps [23].

The inhibitory effect of catechin against the α -glucosidase enzyme has been documented before; however, the advanced purification method used, with a greater percentage of yield, is novel.

To the best of the authors' knowledge, the current study is the first one which was carried out with the intent of isolation of the therapeutically active compound from the *A. unedo* plant roots, and to find out the best method for isolation of catechin from this plant. Moreover, this investigation approved the traditional antidiabetic use of *A. unedo* plant roots. Furthermore, in vivo trials are required to support this therapeutic use and to design a suitable dosage form to produce new drugs or supplements from *A. unedo* plant roots extract, to help in reducing blood glucose level and its complications.

4. Materials and Methods

4.1. Plant Material

The *A. unedo* roots were collected at Beni Mellal region, Morocco, in October 2016. The voucher specimen has been deposited in the Herbarium of the Botany Department at the Scientific Institute of Rabat/Morocco and then voucher specimen code is (RAB 101548). The roots were naturally dried in the shade at room temperature for 2–3 weeks.

4.2. Extraction and Isolation Procedures

The dry roots of *A. unedo* plant were mechanically powdered, and 30 g of the plant material was extracted with 200 mL of water at 100 °C under static nitrogen pressure (100 bars) using the Dionex-ASE (accelerated solvent extraction) prototype Zippertex. The obtained aqueous extract was evaporated using a Rotavap at 100 °C, and lyophilized to give a brown powder which produced 7.12 g of yield (23.7%). After that, 1 g of the obtained extract was adsorbed by the amberlite XAD-16 (Sigma, Steinheim, Germany), which was previously washed with methanol. The pharmacological active

molecule catechin was isolated from roots of *A. unedo* by a methanol-resin reagent, and the yield was 95 mg with 1.33% of the total aqueous extract [32].

4.3. Analytical Methods

4.3.1. Analytical HPLC

An Alliance® Waters W2695 HPLC chain (Waters Corporation, Milford, MA, USA) equipped with a Waters 2996 PDA detector (Waters Corporation, Milford, MA, USA) equipped with a Sunfire III C18 (4.6 mm × 150 mm) 3.5 µm (Waters) reverse phase column. This chromatographic system is coupled to a Waters 2424 light scattering (DEDL) detector. The HPLC system is controlled by Empower 3 software (Waters) (Waters Corporation, Milford, MA, USA). While ultrapure water (MilliQ), 0.1% formic acid/acetonitrile and 0.1% formic acid were the used solvents. In addition, the gradient was from 0 to 100% acetonitrile in 40 min and 10 min to 100% acetonitrile with flow rate of 0.7 mL/min [7].

4.3.2. HPLC Semi-Preparative

The pharmacological active molecule of *A. unedo* roots was isolated by a methanol-resin reagent (95 mg) was identified by preparative HPLC chain equipped with a Waters 717 auto-sampler, a Waters 600 pump, a Waters 2998 DEDL, Waters 2420, PDA detector, and a Waters AF degasser. The column used is a Sunfire III C18 (10 mm × 250 mm) 5 µm. The HPLC system is controlled by Empower 2 software (Waters) (Waters Corporation, Milford, MA, USA). While ultrapure water (MilliQ), 0.1% formic acid/acetonitrile and 0.1% formic acid were the used solvents with a flow rate of 4 mL/min [7].

4.3.3. NMR and HRMS Analysis

The ¹H NMR (300 MHz) and ¹³C NMR (75 MHz) spectra were recorded on Bruker spectrometer with chemical shift values (δ) given in part per million (ppm) relative to TMS (0.00 ppm) and using MeOD as a solvent, the coupling constants (J) are expressed in hertz (Hz) and singlet (s), doublet (d), and doublet of a doublet (dd) as well as the multiplet (m). The high-resolution mass spectra (HRMS) analysis was performed in a negative mode in full mass scan (*m/z* 100 to 600 amu) using a Thermo Scientific Orbitrap Mass Spectrometer Exactive equipped with a heated electrospray ionization source (HESI), and the used resolution was 1000 [22,33].

4.4. Enzyme Inhibition Assay

The α-glucosidase inhibition assay was performed according to the slightly modified method of Kee et al. [34], with some modifications. Briefly, α-glucosidase enzyme (0.1 U/mL) (Sigma-Aldrich, Lyon, France) and substrate *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG, 1 mM) (Sigma-Aldrich, Lyon, France) were dissolved in potassium phosphate buffer (0.1 M, pH 6.7), and all samples were dissolved in distilled water. The inhibitor (150 µL) was pre-incubated with the enzyme (100 µL) at 37 °C for 10 min, then a substrate (200 µL) was added to the reaction mixture. The enzymatic reaction was performed at 37 °C for 30 min. The reaction was then terminated by the addition of Na₂CO₃ (1 M, 1 mL) (Sigma-Aldrich, Lyon, France). All the samples were analyzed in triplicate with different concentrations to determine the IC₅₀ values, and the absorbance was recorded at 405 nm. The α-glucosidase inhibitory activity was expressed as the percentage of inhibition, and the IC₅₀ values were determined. Acarbose (Sigma-Aldrich, Lyon, France) was used as the positive control. The results were expressed as percentage inhibition and calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{(A_c - A_{cb}) - (A_s - A_{sb})}{A_c - A_{cb}} \times 100$$

where *A_c* refers to the absorbance of the control (enzyme and buffer), *A_{cb}* refers to the absorbance of the control blank (buffer without enzyme), *A_s* refers to the absorbance of the sample (enzyme and inhibitor), and *A_{sb}* is the absorbance of the sample blank (inhibitor without enzyme).

4.5. Statistical Analysis

Determination of α -glucosidase inhibitory activity was carried out in triplicate for each sample. The obtained results were presented as means \pm standard deviation (SD), and were then compared using an unpaired *t*-test.

5. Conclusions

The utilized Zippertex technology of extraction with XAD-16 resin had offered the maximum qualitative and quantitative recovery of catechin, with minimum operations, handling, minimum solvent, and time in comparison with other used methods of extraction and isolation. Moreover, the α -glucosidase inhibitory activity of catechin isolated from the *A. unedo* roots exhibited a potential effect in comparison with commercially available α -glucosidase inhibitory agent acarbose which explains their traditional use as a hypoglycemic plant. These findings further imply that catechin or *A. unedo* root extract separated by Zippertex technology can be used as a potential antidiabetic agent.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2223-7747/7/2/31/s1>, Figure S1: ^1H NMR spectra analysis, Figure S2: ^1H NMR spectra analysis, Figure S3: ^1H NMR spectra analysis, Figure S4: ^{13}C NMR spectra analysis, Figure S5: ^{13}C NMR spectra analysis, Figure S6: ^{13}C NMR spectra analysis.

Acknowledgments: The authors wish to thank Mohammed V University in Rabat for its support to carry out this work.

Author Contributions: M.E.A.F., N.J. and H.N.M. designed and conceived the experiments; H.N.M. and W.O. performed the experiments; I.F. conducted the spectral NMR analysis; Y.C. contributed reagents and chemicals; N.J. wrote the paper; S.J. and S.A. revised the manuscript.

Conflicts of Interest: The authors declare that there are no conflicts of interest.

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