Preparation of superfine cinnamon bark nanocrystalline powder using high energy ball mill and estimation of structural and antioxidant properties.

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Abstract: Application of Food material for medicinal use is become a common and safe approach to treat various diseases. Although Nanoscience is seeming to most promising area to be explore at every aspect of existing science including medicine and pharmaceuticals. Medicinal properties and application of various spices are well explored science but their nanopowder synthesis and effect is not very much known. In this present work, we have used commonly known spice from Indian kitchen know as Cinnamon, for synthesis of nano powder high energy ball mill instrument was used. The crystallographic study, functional group analysis, were done using modern characterization equipment such as XRD (X-ray diffraction), FTIR (Fourier transform infrared spectroscopy), SEM (Scanning Electron Microscope), and UV-Visible spectroscopy. XRD measurement confirm that crystal structure of powder milled for 5 hours and 10 hours were different. Similarly, morphology of differently milled sample found to be different from general Cinnamon powder. This might be due the formation of different fractions of particles were formed as a result of deterioration of cohesion bond due to high energy milling.

The present study suggested that cinnamon superfine powder could be a potential source of natural antioxidant and thus could be useful as therapeutic agents and also open new window for the progress of surface science of food materials, which are beneficial for biomedical engineering, pharmaceutical, health, and medicine industries.

Graphical Abstract

Keywords - Cinnamon, Fine powder, Nanoparticle, Crystal structure, Antioxidant Properties.

Introduction

Herbal products are compounds produced by plants that have numerous nutritional and medicinal properties for the development of drugs and healthy food. The food powders are being produced by different pharmaceutical industries and are consumed by mass people across the globe. The knowledge of active functional ingredient at the nanoscale and their antioxidant behavior was not reported at large to the best of my knowledge. The bark of cinnamon is widely used as a powerful spice all over the world from the very start of human civilization as a crude powder [1-3]. Cinnamon bark powder is used not only for cooking and flavoring agent but also for conventional and contemporary medicines. This is also used in fragrances and essence industries. Due to its pungent smell, it is used for different varieties of foodstuffs products. The active component of cinnamon is cinnamaldehyde, which is responsible for its aroma and flavor cinnamon. While cinnamic acid,
eugenol, and coumaric acid cinnamyl alcohol are active components, responsible for the antioxidant, anti-fungals, anti-inflammatory, antipyretic, properties [4-5]. Some researchers also reported that it act as anti-diabetic, anticancer, and memory enhancer [5]. It enhances glucose uptake by activating the insulin receptor kinase activity, auto-phosphorylation of the insulin receptors. In this present study, we focused on the different structures (morphological properties) of Cinnamon food powder and their antioxidant behaviour. Such Food source obtained from the bark as medicine is widely used in recent days as it fewer side effects as compared to artificially synthesize drugs. Nanomaterials, Nanoscience, and nanotechnology are the newly emerging field of nanomedicine. Antioxidant activities, such as total phenolic content, OH scavenging rate, superoxide scavenging ability, are also measured. Nanoparticles usually have improved or different properties than the bulk/crude size of material of the same substance and have different properties and an antioxidant behaviour. For centuries, Cinnamon has also been used as a health-promoting agent for the treatment of diseases. Very Small amounts of cinnamon bark nanoparticles can contribute to being a potent antioxidant [7]. We have tried to address the possible scientific aspect for the industrial production of superfine cinnamon bark powder for its numerous applications. The main objective of this present research is to achieve an understanding about change in structural parameters and morphology of cinnamon bark powder during whole process of grinding by high energy ball milling and their potential applications in food science, pharmaceutical, and Biomedical sectors. [8-10].

Material and Methods

Materials required

In this present research for antioxidant property evaluation, we used, Ethanol, Tris-HCl buffer, DMSO, Gallic Acid, Folin-Ciocalteu reagent, Hydrogen peroxide, pyrogallol (1, 2, 3-trihydroxybenzene)

Preparation of Cinnamon bark Powder using Ball Mill

The high energy Ball milling is the equipment in which the powdered sample of cinnamon bark sample was placed in the stainless-steel jar of 50ml with steel ball 5 mm sizes. The sample of cinnamon bark was powder was prepared using a kitchen based mixture grinder, for size reduction and this powder is called as general/crude powder of cinnamon (say, C0). The ratio of weight of balls to powder is at 20:1 in the 50 ml stainless-steel jar was filled to about 1/3 of its capacity by volume. During the process of milling, the balls were rotated horizontally with constant speed of 500 rpm for up to 5 hours (say, C5) and 10 h (say, C10), respectively. The rotational direction of ball milling was changed after every 30 min interval and a constant temperature at 25°C was maintained by an air-cooling system to balance the overheating of ball milling equipment. Similar method was also used earlier to prepare turmeric, bitter guard, Ginger etc. [11,12,13]. Physical properties of Cinnamon bark fine particles were measured using X-ray diffraction, Scanning electron microscope, Fourier transform infrared spectroscopy, and UV-Visible-NIR spectrophotometer. Biomedical investigations such as Total phenolic content and antioxidant etc. were carried out to determine its potential application. The Structural, morphological, the functional group determination and other properties like antioxidant are discussed in detail in the result and discussion in later part of the journal.

Total phenolic content and antioxidant activity

Total Phenolic Content finding

The total phenolic content of Cinnamon crude powder and nanocrystalline powder was obtained using Ultraviolet-Visible spectrophotometer technique. The Folin-Ciocalteu reagent method has been used [16]. The concentration used for working of prepared food powder was taken as 1mg/ml and 100μg/ml and dissolved in Dimethyl sulfoxide. The absorbance of the solution was obtained at the radiation of a wavelength of 750 nm [13]. The total phenolic content (TPC) was found, using the formula

\[
TPC = \frac{(X-V-DF)}{M}
\]
Where symbol X represents the concentration in ppm, V is the volume of a solution of extract in unit ml, DF = Dilution Factor of the sample solution, and M is the mass of the sample in gram.

**Hydrogen Peroxide scavenging Activity assay**

The fundamental scavenging process of each extract solution was obtained using the H$_2$O$_2$ process [14]. About 2 mL of extract liquid solution (10–100 μg/mL) in methanol was mixed to 4.0 mL of H$_2$O$_2$ (20 mM) solution in phosphate buffer. The pH of the buffer solution was maintained at a numerical value of 7.4. Then at an interval of 10 min, the optical absorbance was found of radiation of wavelength (λmax) 230 nm. This radiation absorbance was noted not in favour of the phosphate buffer blank solution. The scavenging percentage of H$_2$O$_2$ was determined using the equation:

\[
\% \text{scavenging of H}_2\text{O}_2 = \frac{A_0 - A_1}{A_0} \times 100
\]

In this equation symbol, A$_0$ represents the absorbance of the control. The control was taken as phosphate buffer with Hydrogen peroxide. The symbol A$_1$ represents the absorbance of the extracts.

**Superoxide radicals scavenging activity**

In this present experiment, the original pyrogallol (1, 2, 3-trihydroxy benzene) technique was used for superoxide dismutase. This is generally used for measuring superoxide-scavenging of other antioxidants also [15-16]. The procedure of these experimental activities is explained further. A pyrogallol solution (in 1 M HCl) is thoroughly mixed with pH 7.4 Tris-HCl buffer. Again concentration of pyrogallol (25mM) is added in the reaction solution, in which •O$_2$- were scavenged. Further optical absorbance at wavelength 325 nm is measured every 30 seconds for 5 min at 37 °C. The advanced pyrogallol method is a reliable and economic superoxide-scavenging assay suitable for all types of antioxidants.

**RESULTS AND DISCUSSIONS**

**X-ray diffraction (XRD) Measurement**

Structural analysis for synthesized Cinnamon bark food powder were considered using X-ray diffractometer (D8 Advance, Bruker Germany) work at 40kV, 40mA and at room temperature. The diffraction pattern were analysed in range from 10 degrees to 50 degrees at a rate of 2° /min scanning rate, which is been presented in figure 1.

![Figure 1](image1.png)

Figure.1 XRD pattern of Cinnamon powder

Mean crystalline size corresponding to different peaks were determined using Scherrer’s equation [17].

\[
D = \frac{0.94\lambda}{\beta \cos\theta}
\]  

(1)
Where \( D \) = crystalline size, \( K \) (form factor=0.9) \( \beta \) (FWHM) which is equal to twice the diffraction angle \( \theta \), and \( \lambda \) = wavelength for Cu-K\( \alpha \) radiation having value 1.54056\( \AA \).

**Table 1. XRD data of general Cinnamon powder prepared by mixture grinder (C\textsubscript{0})**

| 20 of Peak (Degree) | FWHM | d-spacing (Å) | Size of crystals (nm) |
|---------------------|------|---------------|-----------------------|
| 15.19               | 0.12 | 5.82          | 121.56                |
| 15.55               | 0.12 | 5.69          | 121.51                |
| 24.65               | 0.19 | 3.60          | 49.04                 |
| 28.43               | 0.28 | 3.13          | 28.55                 |
| 30.33               | 0.14 | 2.94          | 79.99                 |
| 31.63               | 0.38 | 2.82          | 20.55                 |
| 34.94               | 0.67 | 2.56          | 10.99                 |

**Table 2. XRD data of Cinnamon powder milled for 5hr using Ball milling (C\textsubscript{0.5})**

| 20 of Peak (Degree) | FWHM | d-spacing (Å) | Size of crystals (nm) |
|---------------------|------|---------------|-----------------------|
| 15.21               | 0.4800 | 5.81          | 79.56                 |
| 24.34               | 0.5760 | 3.65          | 79.46                 |
| 28.18               | 0.0480 | 3.16          | 36.07                 |
| 30.73               | 1.1520 | 2.90          | 28.69                 |
| 31.38               | 1.1520 | 2.84          | 29.40                 |
| 39.15               | 1.1520 | 2.35          | 27.78                 |

**Table 3. XRD data of Cinnamon powder prepared by 10hr using Ball milling (C\textsubscript{1.0})**

| 20 of Peak (Degree) | FWHM | d-spacing (Å) | Size of crystals (nm) |
|---------------------|------|---------------|-----------------------|
| 15.59               | 0.14 | 5.81          | 79.54                 |
| 22.62               | 0.96 | 3.92          | 7.31                  |
| 24.69               | 0.14 | 3.60          | 79.48                 |
| 31.06               | 0.28 | 2.87          | 28.45                 |
| 40.15               | 0.28 | 2.24          | 29.43                 |
| 43.83               | 0.88 | 2.06          | 29.81                 |

The structural data obtained from XRD for general/crude Cinnamon powder, for zero hour milled let it denote by CV\textsubscript{0} (conventional powder obtained by general grinding) which is been shown in table-1. Similarly (CV05) and (CV10) when milled by using high energy ball at 5hr and 10hr respectively in Table 2-3. Further structural parameter was evaluated for above mentioned samples were evaluated like interplanar distance, FWHM, crystallite size and diffraction angle by using Origin 8.0 software. The crystallite size was found to be in range of 29-79 nm, 27-79 nm and 10.99 nm -121.56 nm for 10hr, 5hr and zero hour milled sample respectively. The above result reflects that milling time plays a vital role in crystal structure of nanopowder. Table (1-3) shows the values of different structural parameters like d-spacing, peak positions etc for different hours milled samples respectively, which may indicate that considerable changes in the crystal structure of Cinnamon powder, and distortion of the cell wall structure of powder. Some of the diffraction peaks disappear in 5hr and 10hr nanopowder. XRD analysis also shows that at high milling rate say 5 hrs and 10 hrs some peaks disappears showing amorphous nature of cinnamon (Figure 3 and Table 1-3). Peak intensity did not
change significantly, and this shows the crystallinity of cellulose of materials was not destroyed at large. Thus, angular peak position with the different crystalline size of ground powder for 0hr, 5hr, and 10 hr does not significantly change. This result reveals that some of the crystalline regions were destroyed during grinding for different time duration. Such XRD details of some food powders are also reported [18]. The intense peak at 15° and 24°, which might be due to the presence of crystalline cellulose having assigned planes of (110) and (200). Crystalline size of few crystal of general Food powder (Fig-1 and Table-1) is above 100nm. But when the same powder was milled for 5hr and 10 hr respectively, sizes reduced to less than 100nm (table 2-3). This shows the more grinding produces superfine nanoscale powder.

XRD analysis also shows that at high milling rate say 5 hrs and 10 hrs some peaks disappears showing amorphous nature of cinnamon.

**FTIR analysis**

Functional group of different sizes of Cinnamon bark powder were measured using Fourier Transform Infrared Spectroscope, and pellets were prepared using potassium bromide (KBr) as a binder, Spectra were observed with (PerkinElmer UK) at room temperature.

FTIR spectra of ball-milled cinnamon powder is been shown in fig.2 and data are interpreted in table 4 at different durations of (a) 0 h (b) 5 h (c) 10 h. The FTIR spectra were recorded in range of 400 to 4500 cm⁻¹.

![FTIR spectrum for Cinnamon powder samples](image)

**Table-4:** Details of wave number and Functional group of different Food powder

| FTIR spectra of | C₀ | FTIR spectra of | C₅₁ | FTIR spectra of | C₁₀ |
|----------------|----|----------------|-----|----------------|-----|
| Wavenumber     | C₀ | Wavenumber     | C₅₁ | Wavenumber     | C₁₀ |
| (Cm⁻¹)         |    | (Cm⁻¹)        |     | (Cm⁻¹)        |     |
| 3299 Cm⁻¹      | O-H| 3400 Cm⁻¹      | O-H | 3400 Cm⁻¹      | O-H |
| 2930 Cm⁻¹      | N-H| 2936 Cm⁻¹      | N-H | 2936 Cm⁻¹      | N-H |
| 2058 Cm⁻¹      | N=C=N| 2065 Cm⁻¹ | N=C=N| 2059 Cm⁻¹ | N=C=N |
| 1619 Cm⁻¹      | C=N| 1620 Cm⁻¹      | C=N | 1620 Cm⁻¹      | C=N |
| 1515 Cm⁻¹      | N-O| 1516 Cm⁻¹      | N-O | 1516 Cm⁻¹      | N-O |
| 1450 Cm⁻¹      | CH₃| 1449 Cm⁻¹      | CH₃ | 1451 Cm⁻¹      | CH₃ |
| 1375 Cm⁻¹      | C-H| 1378 Cm⁻¹      | C-H | 1378 Cm⁻¹      | C-H |
| 1317 Cm⁻¹      | NO₂| 1318 Cm⁻¹      | NO₂ | 1318 Cm⁻¹      | NO₂ |
| 1248 Cm⁻¹      | C-O| 1246 Cm⁻¹      | C-O | 1246 Cm⁻¹      | C-O |
| 1157 Cm⁻¹      | C-O-C| 1157 Cm⁻¹ | C-O-C| 1154 Cm⁻¹ | C-O-C |
| 1020 Cm⁻¹      | C-OH| 1017 Cm⁻¹     | C-OH | 1017 Cm⁻¹     | C-OH |
| 894 Cm⁻¹       | C-Cl| 896 Cm⁻¹       | C-Cl | 896 Cm⁻¹      | C-Cl |
| 782 Cm⁻¹       | C-H| 782 Cm⁻¹       | C-H | 780 Cm⁻¹      | C-H |
| 517 Cm⁻¹       | C-Br| 515 Cm⁻¹     | C-Br | 515 Cm⁻¹ | C-Br |

FTIR spectra of ball-milled cinnamon powder is been shown in fig.2 and data are interpreted in table 4 at different durations of (a) 0 h (b) 5 h (c) 10 h. The FTIR spectra were recorded in range of 400 to 4500 cm⁻¹.
4000 cm$^{-1}$ wave number. Table 4 depicts the materials present in the prepared material. The results show that there is no new chemical bond was found. Though, very few of characteristics bonds were found to change in transmittance or absorbance range. Further result shows that crystal size is not the function of functional group. Grinding couldn’t effect the intramolecular structure of the prepared material significantly. This can only destroys the cohesive amorphous region of surface of crystallites. The chemical bond present show the presence of cellulose in cinnamon powder [3, 7]. Such research were also found by other, shows change number of bond, surface area, density, porosity changes, as reported by some research group [19]. SEM morphology supports these findings, which are shown in figure-3.

**Surface morphology of Cinnamon powder**

Scanning electron microscope (EVO 18 Zeis UK) was used to study the morphology of synthesized material. The standard parameters were maintained i.e sample were analysed at 20 KV. The sem images of different milling our sample is been shown in Fig 3(a-c). External surface morphologies and large agglomerations of particles show that molecular structure is distortion shape of different micron sizes Further the result shows large agglomerations and distorted shape of micron size. Milling hours also effect the morphology of the prepared material. This observation shows that griding is also effective in changing the original structure of cinnamon powder. Surface morphology after milling at different hr. changed significantly, which can have a considerable impact on Physical-Chemical behaviour for various industrial applications. High energy Milling weakened the cohesive force and broke the particles into different size fractions, and they combined aggregation results of various shapes of particles, as shown in fig.3 (a-c). Different surface structures were also reported by some of the research groups by pressure grinding/ball milling [20].

**Figure 3. (a-c) - SEM images for prepared Cinnamon Nanopowders**
UV-VIS NIR Measurement

The UV-visible (Lambda 950, PerkinElmer, UK) spectrum were analysed in the wavelengths 200 to 550 nm and results are shown in figure 4. Proteins biomaterials are present in animal tissue and plants that strongly absorb radiation in the range of about 280 nm [21-22]. It may be due to the amino acids, which made up the proteins and absorb the radiation in UV range. Such type of UV absorption can be used for the identification of protein bands in the food materials. In the present research, we have taken food nanoparticles (Cinnamon), and it has found maximum absorbance in the range of 280 nm radiation. The spectra also reveal that as the milling hours are increased, it also shows more absorption. This study reveals that absorption depends on the degree of superfine powder.

![Figure 4: UV-VIS-NIR spectrum of Ginger Cinnamon powder.](image)

ANTIOXIDANT PROPERTIES

Total phenolic content

In antioxidant activities, Phenolic compounds exhibit redox behaviour. The free radical activities are due to the hydroxyl group, which are present in the materials. As mentioned in the materials method section that phenolic content was calculated by the Folin–Ciocalteu reagent method [13]. The experimental findings were obtained from a calibration curve of equations

\[
y = 0.0097X - 0.3967, \quad R^2 = 0.9865
\]

of gallic acid. The range of concentration is taken between 0 – 450 μg/mL and articulated in gallic acid equivalents (GAE) per gram dry extract weight (Table 5 and fig-5). In Which Y is the absorbance, X is the total phenolic content, and R square is regression coefficient. R^2 is statistically measured that reflects the proportion of the variance for a dependent to relative variable. R squared shows to what extent the variance of one variable explains the variance of a second.
### Figure 5: Total phenolic content standard curve with reference to gallic acid.

![Graph showing total phenolic content and gallic acid content](image)

### Table 5- In vitro antioxidant activity assay and Total Phenolic Contents (TPC) of superfine cinnamon particles (Value of mean ±SD of up to 3 replications)

| Details( group)                          | Total phenolic content in mg, GAE/g( ±SD) | Hydroxyl radicals % scavenging activity±SD) | Superoxide radicals % scavenging ability assay±SD) |
|------------------------------------------|------------------------------------------|---------------------------------------------|--------------------------------------------------|
| General Cinnamon powder(1mg/ml) in DMSO  | 49.58 ± 0.077                            | 21.69 ± 1.72                                | 33.9 ± 0.22                                      |
| Superfine Cinnamon powder of 5hr milling (1 mg/ml) in DMSO | 49.69 ± 0.070                            | 37.6 ± 1.28                                 | 45.52 ± 1.66                                    |
| Superfine Cinnamon powder of 10hr milling(1mg/ml) in DMSO | 60.28 ± 0.18                            | 61.47 ± 0.64                                 | 56.44 ± 1.66                                    |
| General Cinnamon powder(100μg/ml) in DMSO | 22.68 ± 1.03                            | 16.52 ± 1.51                                 | 21.22 ± 0.82                                    |
| Superfine Cinnamon powder of 5hr milling(100μg/ml) in DMSO | 37.68 ± 0.52                            | 26.57 ± 1.25                                 | 40.76 ± 0.67                                    |
| Superfine Cinnamon powder of 10hr milling(100μg/ml) in DMSO | 44.94 ± 0.37                            | 49.15 ± 2.27                                 | 47.7 ± 0.20                                     |

Superfine Cinnamon bark powder has total phenolic content ranges amid 49.58 mg to 60.28 mg G/g for crude /general Cinnamon powder (C₀), (C₅), (C₁₀) prepared food powder materials for the concentration of the sample 1mg/ml, whereas Total Phenolic Content value ranges are 22.68 to 44.94 GAE/g for concentration 100 μg/ml of cinnamon superfine powder. The outcome shows that total phenolic contents concentration depend in present materials and surface structure of food Nanopowders [table-5]. This measurement shows that surface science and related crystal structure changes the TPC activities significantly. In this present study, Surface reactivity was found to increase for three prepared materials.
Free radical scavenging

a) Hydroxyl radicals scavenging activity

![Graph showing Hydroxyl radical scavenging activity](image)

**Fig. 6** - Effect of cinnamon powder on the Hydroxyl radial scavenging effect (Value of mean ±SD of 3 replications)

The in vitro analysis of antioxidant capability of general/crude Cinnamon powder (C₀) and Cinnamon superfine powder after five hours of milling (C₅) and 10 hours of milling powder (C₁₀) was enhancing as the size of the particle and surface area of the material changes. The scavenging activity was highest i.e. 61.47 ± 0.64 % found in the sample which is milled for 10 hours with the concentration of working solution was 1mg/ml DMSO. Whereas the scavenging capability of crude/general Cinnamon powder at this concentration is attain as 21.59 ± 1.72%, which is quite low as compared to superfine cinnamon powder, as given in figure 6 and table 5. While for the concentration of 100μg/ml, largest scavenging was obtained 49.15± 2.27 % and minimum scavenging was found 16.52 ± 1.51 % for 10hr milled and 0 hr milled Cinnamon food powder.

Pyrogallol superoxide radicals scavenging activity

![Graph showing Pyrogallol superoxide scavenging activity](image)

**Figure7.** In vitro Superoxide scavenging activity of different sizes of Cinnamon powder of different size and concentrations in DMSO.

The superoxide emitted, by some Biological & Photochemical reactions and attacking important biological organs is a toxic species. The elemental oxygen and OH radical is the constituent of superoxide radical which may increases oxidative stress, pathological incident, destructions of cell, etc. These radicals may also produced by auto-oxidation. In the current research study the scavenging
activity of superfine Cinnamon powder was recorded, through spectrophotometer and data obtained in this process shows that the binding capacity to the \( O_2 \) radicals generated by the metabolic reaction and is highest in 10 hours of milled superfine cinnamon bark powder when 1mg of powder dissolved in 1ml DMSO is 53.9 ± 0.0012 % as shown in figure 7 and table 5. Whereas the scavenging capability at 0 hours milled fine cinnamon bark powder (1mg/ml) in DMSO is 39.2 ± 0.0037 %. (Value of mean ±SD of 3 replications. Thus, from table-5, fig.6-7, we found that Hydroxyl radicals and superoxide scavenging ability increases with increase in concentration and decrease in size of superfine cinnamon powder. Therefore, present studies suggest that medicinal plant products like spices condiments, fruit seed powder could be engineered for commercially processing and production of food Nanopowders which is seemed to be a promising good effect on health and could be used as new functional materials by pharmaceutical sector and Biomedical Engineering. Such antioxidant capability help improve the nutritional value of prepared food materials. Based on reported work, In a nutshell, it may be concluded that the major effect in the present studies is that the grinding produces a small crystalline size of cinnamon food particles. Reduction in size increases surface reactivity and agglomerations and increases the efficiency of antioxidant properties. Similar results are also reported for green tea powder and other food powder [23-25]

CONCLUSIONS

Superfine Cinnamon food Nanocrystalline powder of different morphology and crystal structures were successfully prepared using high energy ball milled for prominent industrial and scientific significance. The crystal structure, functional group, were evaluated using modern scientific tools such as X-Ray Diffraction (XRD), Fourier Transforms Infrared Spectroscopy (FTIR), Scanning Electron Microscope(SEM) and found considerable change in position of wave number, inter-planar distance and reactivity’s at the surfaces. The present study reveals grinding produces a new surface structure, which is beneficial for physicochemical behaviour. Optical measurement shows that UV absorbance was found at about 280nm. The phenolic content, Hydroxyl radicals and superoxide radicals scavenging activity were found to increase as the milling hour, and superfine behaviour increases. Antioxidant properties also depend on the concentration of food powder. The current research finding opens a new aspect for the development of surface science of food nanopowders and its potential biomedical applications, drug discovery, health sciences, and medicine industries.

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