Extractive Values and Antioxidant Properties of Leaves, Seeds, Pods and Coats Moringa Plant

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ABSTRACT

The need for identifying and using effective solvents for extraction of bioactive ingredients of high antioxidant properties from plant sources is paramount. The aim of this work is to establish the best solvent for extraction of bioactive ingredients as well as the part of moringa plant that is richest in antioxidants properties. The seeds, leaves, pods and coats of moringa plant were obtained, cut, ground and sieved with 40 mm mesh and separately extracted using six different solvents (acetone, ethyl acetate, methanol, ethanol, water and chloroform) at ratio 1:10 for 72 h. The efficiency of each solvent was determined as percent extractive value. The first two highest solvent extracts for each of the seeds, leaves, pods and coats of moringa plant were analyzed for antioxidant properties. It was observed that moringa seeds had the highest extractive values in all the solvents used while moringa pods had the lowest extractive values. The solvent extraction efficiency decreases in order of acetone, ethyl acetate, ethanol, methanol, water and chloroform. There was significant difference at p<0.05 in the extractive values of the seeds, leaves, pods and coats of moringa plant in all the solvents used. There were significant differences at p < 0.05 in the total flavonoid, total phenol, DPPH, iron chelation assay and ferric reducing antioxidant power of raw sample, ethanol extract and ethyl acetate extract of moringa leaves as well as all the antioxidant properties for raw sample, acetone extract and ethanol extract of moringa coats. Seeds and leaves of moringa were richest in antioxidant properties.

Introduction

Antioxidants are molecules that fight free radicals in human body [1]. Antioxidants cause protective effect by neutralizing free radicals which are toxic byproducts of natural cell metabolism. Research is increasingly showing that antioxidant rich foods and herbs have health benefits. Medicinal herbs are the richest sources of antioxidants compounds [2]. Antioxidants are biomolecules which tackle and destroy free radicals and scavenge diseases [3]. Sagar, et al. and Shao, et al. reported that green plants are chief sources of natural antioxidants, and they are capable of tackling free radicals [3-5]. Moringa oleifera belongs to the single genus family Moringaceae and it is most studied of the thirteen species of Moringa trees [6,7]. It is a deciduous tree that grows up to 12 m tall with an umbrella-shaped crown and grows extremely fast which can reach up to 4 m in its first year. Its leaves are alternate bi or tri-pinnate, 20-70 cm long. Leaflets are usually oval, rounded at the tip, and 1-2cm long and they are dark green in colour with almost whitish in the lower surface [8,9].
46 antioxidants, magnesium and protein among other nutrients. The plant is called “Zogale” in Hausa, “Ewe Igbele” in Yoruba, “Ikwe Oyibo” in Ibo and “Egelengedi” in Idoma while its English name is Bean oil tree or drumstick tree or miracle tree or “Mother’s Best Friend” [6,10,11]. Though a lot of research works have been done on moringa plant [10,12-16], but there is little, or no work done on the effect of solvents in extracting phytochemicals from the seeds, coats, pods and leaves of the plant. Therefore, the focus of this research work is to evaluate the potency of solvents in extracting bioactive ingredients from seeds, coats, pods and leaves of moringa plant as well as to investigate and compare the antioxidant properties of the first two highest yield solvent-extracts with that of the raw sample with a view of establishing which of the moringa plant parts (seeds, leaves, pods and coats) is richest in phytochemical constituents and antioxidant properties.

Materials and Methods

Source of Materials

The seeds, coats, pods and leaves of Moringa oleifera were collected from a compound of a building at Ajagbale Street, Oka, Ondo City, Ondo State, Nigeria. All chemicals used were of the analytical grade with the highest purity available (<99.5%) and procured from Sigma Aldrich, USA.

Preparation and Extraction of Seeds, Coats, Pods and Leaves of Moringa Plant

The different parts of moringa plant were cut into smaller pieces for easy air-drying. The dried samples were ground separately using electric blending machine (Solitare Mixer Grinder VTCL Heavy Duty 750 Watts) and each part was sieved with 40 mm mesh size. The powdered samples were divided into portions, packed in air tight containers labelled appropriately prior to extraction. Each sample was extracted separately with each solvent (acetone, chloroform, ethyl acetate, ethanol, methanol and water) at ratio 1:10 for 72 h during which it was intermittently shaken on a shaking orbit machine. The resulting mixture was filtered through a 0.45 μm nylon membrane filter. The extracts were desolventised to dryness under reduced pressure at 40 °C by a rotary evaporator (BUCHI Rotavapror, Model R-124, Germany). Weight of extract obtained was used to calculate the percentage yield of extract in each solvent and the dry extracts were stored in a refrigerator (4 °C) prior to analysis [17-19].

Determination of Antioxidant Property

Total Flavonoid

0.1g of extract was weighed into a sample bottle; 10 mL of 80% methanol was added and allowed to soak for 2 hours. 0.4 mL of the solution was measured into a 10 mL volumetric flask, 1.2 mL of 10% sodium hydroxide, 1.2 mL of 0.2 M concentrated sulphuric acid and 3 mL of 3 M sodium nitrate were added. 4.2 mL of distilled water was used to make it up. The absorbance was read using 6850 UV spectrophotometer at wavelength 325 nm [20].

\[
\text{Total Flavonoid (mg/100g) } = \frac{\text{Concentration in mg/l} \times \text{Volume of Sample} \times \text{DF}}{\text{Sample Weight}}
\]

Ferric Reducing Antioxidant Power (FRAP)

0.1g of extract was weighed into a sample bottle; 10 mL of 80% ethanol was added. 2.5 mL sodium phosphate buffer (0.2 M Na2PO3, pH 6.6) and 2.5 mL of 1% potassium ferricyanide were added and incubated at 50°C for 20 minutes. 2.5 mL of TCA (trichloroacetic acid) was added to stop the reaction. 2.5 mL of the aliquot was taken and diluted with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride was added and allowed to stand for 30 minutes in the dark for color development. The absorbance was read using 6850 UV/Visible spectrophotometer at wavelength 700 nm [21].

\[
\text{FRAP (garlic acid equivalent) (GAE) } = \frac{\text{Absorbance of Control} - \text{Absorbance of Test Sample} \times \text{DPPH Scavenged}}{\text{Volume of Extract} \times \text{DF}}
\]

\[
\text{DPPH Scavenged } = \frac{\text{Absorbance of Control} - \text{Absorbance of Test Sample}}{\text{Absorbance of Control} \times \text{Sample Weight} \times 100}
\]

Total Phenol

0.1g of extract was weighed into a sample bottle; 10 mL of distilled water was added to dissolve. 1 mL of the solution was pipetted into a test tube and 0.5 mL of 2 N Folin-Ciocalteu reagent and 1.5 mL of 20 % sodium carbonate solution was added. The solution was allowed to stand for 2 hours and the absorbance was read using a 6850 UV/Visible spectrophotometer at wavelength 765 nm. Garlic acid solution was used as standard viz 0.5 mg, 1 mg, 2 mg, 4 mg, 6 mg, 8 mg and 10 mg [22].

\[
\text{Phenol content mg/100g } = \frac{\text{Concentration in mg/l} \times \text{Volume of Sample} \times \text{DF}}{\text{Sample Weight}}
\]

DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) Scavenging

0.1g of extract was weighed into a sample bottle and 10 mL of ethanol was added, stirred for 15 minutes and allowed to stand for 2 hours. 1.5 mL of the extract was pipetted into a test tube and 1.5 mL of DPPH solution was added. The 6850 UV/Visible spectrophotometer was zeroed with ethanol as the blank solution. The absorbance/optical density of the control (DPPH solution) was read. The absorbance of the test sample was read at 517 nm. [23].
Iron (Fe²⁺) Chelation Assay

0.1g of extract was weighed into a sample bottle, 150 μL of 500 μM FeSO₄ was added. 168 μL of 0.1M Tris-HCl (pH 7.4) and 218 μL of saline solution was added. 100 μL of the solution was taken and incubated for 5 minutes, before addition of 13 μL of 0.25% 1, 10-phenanthroline. The absorbance was read using 6850 UV/Visible spectrophotometer at wavelength 510 nm [24].

\[
\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Extract}}{\text{Absorbance of Control}} \times 100
\]

Statistical Analysis

Statistical significance tests were performed using SPSS (v. 20, IBM SPSS Statistics, US) at p < 0.05 by means of one-way analysis of variance (ANOVA) followed by LSD post hoc multiple comparison and the experimental results were expressed as mean ± standard deviation of three replicates.

Results and Discussion

Extractive Values of Solvent Extracts of Leaves, Seeds Pods and Coats of Moringa Plant

The extractive values (% yield) of leaves, seeds, pods and coat moringa plant using acetone, chloroform, ethanol, ethyl acetate, methanol and water are contained in Table 1. The result showed that the percentage yield of moringa leaf extract was 11.095±0.805 in ethyl acetate, 9.291±2.234 in ethanol, 8.497±0.713 in water, 8.108±2.219 in methanol, 7.039±2.084 in acetone and 3.055 ± 1.735 in chloroform. The percentage yield of moringa pod extract was 2.146±0.003 in methanol, 1.347±0.001 in ethanol, 1.047±0.000 in ethyl acetate, 0.898±0.002 in chloroform, 0.850±0.000 in acetone and 0.199±0.002 in water. The percentage yield of moringa coat extract was 8.709±0.414 in acetone, 3.333±0.108 in ethanol, 3.160±2.731 in methanol, 3.062±0.534 in ethyl acetate, 2.149±1.799 in water and 1.451±0.502 in chloroform. The percentage yield of moringa seed extract was 30.151±1.221 in acetone, 29.997±1.558 in ethyl acetate, 27.725±2.714 in ethanol, 26.190±3.452 in methanol, 23.942±2.955 in chloroform and 21.417±10.455 in water.

In each of the solvent used, there was a significant difference at p=0.05 in the extractive values of the seeds, leaves, pods and coats of moringa plant. In all the solvents used for extraction, it was observed that the extractive value (%) was highest in moringa seed and least in moringa pod. All the solvents used had the first two highest extractive values in moringa seeds and leaves except acetone that had its first two highest extractive values in moringa seeds and coats. According to Alachaher, et al. 2018 [21], there are quite number of factors in which extraction of bioactive compounds depends. The selection of solvent system largely depends on the specific nature of the bioactive compounds being targeted. Also, different solvent systems are available to extract the bioactive compounds from natural products. Extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle size, the solvent used, as well as the presence of interfering substances. Under the same extraction time and temperature, solvent and composition of sample are known as the most important parameters [21].

Table 1: Extractive value of solvent extracts of moringa plant.

| Solvent Extract | *Extractive Values |
|-----------------|--------------------|
|                 | Moringa leaves     | Moringa seeds      | Moringa pods       | Moringa coats      |
| Acetone         | 7.039±2.084        | 30.151±1.221       | 0.850±0.000        | 8.709±0.414        |
| Chloroform      | 3.055±1.735        | 23.942±2.955       | 0.898±0.002        | 1.451±0.502        |
| Ethanol         | 9.291±2.234        | 27.725±2.714       | 1.347±0.001        | 3.333±0.108        |
| Ethyl acetate   | 11.095±0.805       | 29.997±1.558       | 1.047±0.000        | 3.062±0.534        |
| Methanol        | 8.108±2.219        | 26.190±3.452       | 2.146±0.003        | 3.160±2.731        |
| Water           | 8.497±0.713        | 21.417±10.455      | 0.199±0.002        | 2.149±1.799        |

Note: * = Result values are expressed as mean value of triplicate determinations ± standard mean deviation

Different letter in the same row showed significant difference (p<0.05).

Antioxidant Properties of Solvent Extracts of Leaves, Seeds, Pods and Coats of Moringa Plant

Antioxidant properties were carried out on the plant raw sample and the first two solvent extracts with the highest extractive values. The antioxidant properties of raw sample, ethanol and ethyl acetate extracts of leaves; acetone and ethyl acetate extracts of seeds; methanol and ethanol extracts of pods as well as acetone and ethanol extracts of coats of moringa plant were examined and these are presented in Table 2 to Table 5. The antioxidant properties of raw sample, ethanol and ethyl acetate extracts of moringa leaves is depicted in Table 2. The concentration (mg/100g) of total flavonoid in moringa leaves ranged between 0.122±0.001 - 0.332±0.001 with ethanol extract had the highest concentration of 0.332±0.001.
mg/100 g, followed by ethyl acetate extract with concentration of 0.268 ± 0.002 mg/100 g while the powdered raw sample has the least concentration of 0.122 ± 0.001 mg/100 g. The total phenol concentration (mg/100 g) in moringa leaves ranged between 0.181 ± 0.002 - 0.349 ± 0.003 with ethanol extract having the highest concentration of 0.349 ± 0.003 mg/100 g, followed by ethyl acetate extract with concentration of 0.251 ± 0.001 mg/100 g while the powdered raw sample had the least concentration of 0.181 ± 0.002 mg/100 g.

Table 2: Antioxidant properties of moringa leaves.

| Antioxidant Properties | Raw Sample | Ethanol extract | Ethyl acetate extract |
|------------------------|------------|-----------------|----------------------|
| Total flavonoid (mg/100 g) | 0.122 ± 0.001 | 0.332 ± 0.001 | 0.268 ± 0.002 |
| Total Phenol (mg/100 g) | 0.181 ± 0.002 | 0.349 ± 0.003 | 0.251 ± 0.001 |
| DPPH (%) | 89.25 ± 0.21 | 63.10 ± 0.30 | 57.45 ± 0.16 |
| Iron (Fe²⁺) chelation assay (%) | 24.95 ± 0.12 | 4.99 ± 0.05 | 22.56 ± 0.10 |
| Ferric reducing antioxidant power (FRAP) (GAE) | 0.32 ± 0.01 | 0.47 ± 0.00 | 0.87 ± 0.02 |

Note: * = Values are expressed as mean value of triplicate determinations ± standard mean deviation; GAE = Garlic Acid Equivalent
Different letter in the same row showed significant difference (p<0.05).

The percentage DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity of powdered raw sample showed the highest value of 89.25 ± 0.21% followed by ethanol extract of 63.10 ± 0.30% while ethyl acetate extract had the lowest value of 57.45 ± 0.16%. The ethyl acetate extract of moringa leaves exhibited the highest reducing power of concentration of 0.87 ± 0.02 GAE followed by ethanol extract which had concentration of 0.47 ± 0.00 GAE while the powdered sample had the least concentration of 0.32 ± 0.01 GAE. There were significant differences at p < 0.05 in the total flavonoid, total phenol, DPPH, iron chelation assay and ferric reducing antioxidant power of raw sample, ethanol extract and ethyl acetate extract of moringa leaves. The antioxidant properties of raw sample, acetone and ethyl acetate extracts of moringa seeds is displayed in Table 3. The total flavonoid concentration (mg/100g) of moringa seeds ranged from 0.363 ± 0.002 - 0.392 ± 0.000 in which the powdered sample had the highest concentration of 0.392 ± 0.000 mg/100g, followed by ethyl acetate extract with concentration of 0.379 ± 0.001 mg/100g while the acetone extract has the least concentration of 0.363 ± 0.002 mg/100g.

Table 3: Antioxidant properties of moringa seeds.

| Antioxidant Properties | Raw Sample | Acetone extract | Ethyl acetate extract |
|------------------------|------------|-----------------|----------------------|
| Total flavonoid (mg/100 g) | 0.392 ± 0.000 | 0.363 ± 0.002 | 0.379 ± 0.001 |
| Total Phenol (mg/100 g) | 0.059 ± 0.001 | 0.349 ± 0.003 | 0.251 ± 0.001 |
| DPPH (%) | 79.81 ± 0.11 | 71.86 ± 0.30 | 77.43 ± 0.16 |
| Iron (Fe²⁺) chelation assay (%) | 22.36 ± 0.11 | 39.72 ± 0.09 | 38.12 ± 0.07 |
| Ferric reducing antioxidant power (FRAP) (GAE) | 0.29 ± 0.01 | 0.54 ± 0.01 | 0.55 ± 0.00 |

Note: * = Values are expressed as mean value of triplicate determinations ± standard mean deviation; GAE = Garlic Acid Equivalent
Different letter in the same row showed significant difference (p<0.05).

There was no significant difference (p < 0.05) in the total flavonoid content of raw sample, acetone extract and ethyl acetate extract of moringa seeds. In moringa seeds, the total phenol concentration (mg/100g) was between 0.059 ± 0.001 - 0.349 ± 0.00. The ethanol extract had the highest concentration while the powdered raw sample had the lowest concentration of total phenol. The ethyl acetate extract of moringa seeds had total phenol concentration of 0.251 ± 0.001 mg/100 g. There was significant difference (p < 0.05) in the total phenol concentration of raw sample, acetone extract and ethyl acetate extract of moringa seeds. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of powdered raw sample of moringa seeds had the highest activity of 79.81 ± 0.11% followed by ethyl acetate extract with DPPH activity of 77.43 ± 0.16% while acetone extract showed the lowest activity of 71.86 ± 0.30%. There was significant difference (p < 0.05) in the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of powdered raw sample of moringa seeds.
activity of raw sample, acetone extract and ethyl acetate extract of moringa seeds. The iron chelation activity (%) ranged between 22.36 ± 0.11 - 39.72 ± 0.09 with the lowest and highest concentration in raw sample and acetone extract respectively while the ethyl acetate had iron chelation activity of 38.12 ± 0.07%.

There was no significant difference (p < 0.05) in iron chelating activity of acetone extract and ethyl acetate extract of moringa seeds. The ethyl acetate extract of moringa seeds exhibited the highest ferric reducing antioxidant power of concentration of 0.55 ± 0.00 GAE followed by acetone extract which had concentration of 0.54 ± 0.01 GAE while the powdered sample had the least concentration of 0.29 ± 0.01 GAE. There was no significant difference (p < 0.05) in ferric reducing antioxidant power (FRAP) of acetone extract and ethyl acetate extract of moringa seeds. The antioxidant properties of raw sample, methanol and ethanol extracts of moringa pods is shown in Table 4. The concentration (mg/100g) of total flavonoid of moringa pods ranged between 0.038 ± 0.000 - 0.082 ± 0.002 with methanol extract having the highest concentration and the powdered sample having the least concentration while the total flavonoid concentration of ethanol extract of moringa pod was 0.059 ± 0.001 mg/100g. There was significant difference (p < 0.05) in total flavonoid concentration of raw sample, methanol extract and ethanol extract of moringa pods. The total phenol concentration (mg/100g) of moringa pods was between 0.031 ± 0.000 - 0.131 ± 0.001.

Table 4: Antioxidant properties of moringa pods.

| Antioxidant Properties | Raw Sample | Methanol extract | Ethanol extract |
|------------------------|------------|------------------|----------------|
| Total flavonoid (mg/100g) | 0.038 ± 0.000 | 0.082 ± 0.002 | 0.059 ± 0.001 |
| Total Phenol (mg/100g) | 0.131 ± 0.001 | 0.059 ± 0.000 | 0.031 ± 0.000 |
| DPPH (%) | 83.75 ± 0.11 | 98.82 ± 0.20 | 81.95 ± 0.10 |
| Iron (Fe²⁺) chelation assay (%) | 28.14 ± 0.01 | 36.73 ± 0.03 | 32.14 ± 0.02 |
| Ferric reducing antioxidant power (FRAP) (GAE) | 0.25 ± 0.00 | 0.53 ± 0.03 | 0.42 ± 0.01 |

Note: * = Values are expressed as mean value of triplicate determinations ± standard mean deviation; GAE = Garlic Acid Equivalent

The highest and lowest total phenol concentration were found in ethanol extract and raw sample of moringa pods respectively while methanol extract had total phenol concentration of 0.059 ± 0.000 mg/100g. There was significant difference (p < 0.05) in total phenol concentration of raw sample, methanol extract and ethanol extract of moringa pods. For DPPH (2,2-diphenyl-1-picyrilhydrazyl) scavenging activity of moringa pods, it was observed that methanol extract showed the highest value of 96.82 ± 0.20% followed by powdered raw sample which had 83.75 ± 0.11% while ethanol extract showed the lowest value of 81.95 ± 0.10%. There was no significant difference (p < 0.05) in DPPH scavenging activity of raw sample and ethanol extract of moringa pods. The iron chelating activity (%) of moringa pods ranged from 28.14 ± 0.01 - 36.73 ± 0.03 with the lowest and highest concentration in methanol extract and raw sample accordingly and that of ethanol extract was 32.14 ± 0.02%. There was significant difference (p < 0.05) in iron chelating activity of raw sample, methanol extract and ethanol extract of moringa pods.

The ferric reducing antioxidant power (FRAP) of methanol extract of moringa pods exhibited the highest reducing power of 0.53 ± 0.03 GAE, followed by ethanol extract of 0.42 ± 0.01 GAE while the powdered raw sample had least concentration of 0.25 ± 0.00 GAE. There was significant difference (p < 0.05) in ferric reducing antioxidant power of raw sample, methanol extract and ethanol extract of moringa pods. The antioxidant properties of raw sample, acetone extract and ethanol extract of moringa coat is presented in Table 5. The total flavonoid concentration (mg/100g) of moringa coats ranged from 0.049 ± 0.000 - 0.317 ± 0.002 with acetone extract had the highest concentration and the powdered raw sample had the least concentration while ethanol extract had total flavonoid of 0.258 ± 0.001 mg/100g. The concentration (mg/100g) of total phenol of moringa coat was between 0.031 ± 0.000 - 0.118 ± 0.002. The acetone extract and ethanol extract had the lowest and highest concentration while the raw sample of moringa coat had total phenol concentration of 0.063 ± 0.001 mg/100g.
Table 5: Antioxidant properties of moringa pods.

| Antioxidant Properties                      | Raw Sample              | Moringa Coats*          | Ethanol extract |
|--------------------------------------------|-------------------------|-------------------------|----------------|
| Total flavonoid (mg/100g)                  | 0.049±0.000             | 0.317±0.002             | 0.258±0.001    |
| Total Phenol (mg/100g)                     | 0.063±0.001             | 0.031±0.000             | 0.118±0.002    |
| DPPH (%)                                   | 85.07±0.09              | 72.13±0.10              | 95.20±0.12     |
| Iron (Fe²⁺) chelation assay (%)            | 30.34±0.02              | 11.18±0.00              | 44.91±0.04     |
| Ferric reducing antioxidant power (FRAP)   |                         |                         |                |
| (GAE)                                      | 0.32±0.01               | 0.85±0.05               | 0.64 b±0.03    |

Note: * = Values are expressed as mean value of triplicate determinations ± standard mean deviation; GAE = Garlic Acid Equivalent

The DPPH of ethanol extract moringa coat showed highest activity of 95.20±0.12 % followed by powdered sample which had activity of 85.07±0.09% while acetone extract showed the lowest activity of 72.13±0.10%. The iron chelating activity (%) of moringa coats was between 11.18±0.00 - 44.91±0.04. Ethanol extract had the highest iron chelating activity while acetone extract had the lowest and that of raw sample was 30.34±0.02 %. The ferric reducing antioxidant power of acetone extract of moringa coats exhibited the highest reducing power of 0.85±0.05 GAE followed by ethanol extract of 0.64±0.03 GAE while the powdered raw sample has the least concentration of 0.32±0.01 GAE. There were significant differences (p < 0.05) in all the antioxidant properties considered for raw sample, acetone extract and ethanol extract of moringa coats [25-28].

Conclusion

The moringa seeds are richest in bioactive ingredients and this is followed by moringa leaves while the least bioactive ingredients are found in moringa pods. The solvent extraction efficiency of bioactive ingredients in moringa plant decreases in the order of acetone, ethyl acetate, ethanol, methanol, water and the least is chloroform. The utilization of acetone, ethyl acetate, ethanol and methanol in extracting bioactive ingredients of high antioxidant activities from the seeds and leaves of moringa plant is economical and effective. Further scientific investigation can be conducted using acetone, ethyl acetate, ethanol and methanol extracts of seeds and leaves of moringa plant as antioxidants or preservative in edible oils and their antioxidative potentials can be compared with synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT) etc. in edible oils.

Conflict of Interest

No conflict of interest with any institution/organization.

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