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## ***In vitro* Antioxidant and Chemical Constituents from the Leaves of *Ormocarpum cochinchinense* Elumbotti**

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### **ABSTRACT**

The phytochemical screening and antioxidant properties of the leaves of *Ormocarpum cochinchinense* L. were studied after extraction of various compounds in it using different solvents. The different solvents used for extraction was, dimethyl sulfoxiamide (DMSO), Ethyl Acetate (EtoAc), ethanol (EtOH), methanol (MeOH) and chloroform (CHCl<sub>3</sub>). Antioxidant potential was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. The DMSO, EtoAc, EtOH, MeOH, CHCl<sub>3</sub> extracts showed significant ( $p < 0.001$ ) antioxidant potentiality in a dose-dependent manner. Its IC<sub>50</sub> values (13.05±0.39; 14.08±0.42; 15.02±0.45; 16.12±0.48; 14.88±0.44 µg mL<sup>-1</sup>) were compared to the IC<sub>50</sub> value of the reference standard ascorbic acid (06.10±0.18; 07.18±0.21; 08.15±0.24; 09.10±0.27; 08.18±0.23 µg mL<sup>-1</sup>) which suggested that the *Ormocarpum cochinchinense* is a potent antioxidant. The phytochemical evaluation indicated the presence of chemical constituents including flavonoids, alkaloids, steroids, terpenoids, saponins, gums, tannins, resins, coumarins, glycosides, carbohydrates. This study also shows that the different solvent extract of leaves of *O. cochinchinense* has bioactivity. Further the compound needs to be isolated to confirm the activities of the individual compounds.

**Key words:** Antioxidant, DPPH, phytochemical, flavonoids, *Ormocarpum cochinchinense*

### **INTRODUCTION**

*Ormocarpum cochinchinense* that belonging to Leguminaceae is a small herb found all over different parts of Tamil Nadu. It is an efficient bone fracture healer popular only to a few villagers in Tamil Nadu and hence it got its name as “Elumbotti”. This herb has been used as source of medicine by human from ancient time to the present day. Most of the medicines in practice were formulated based on plant derived metabolites (Samy *et al.*, 2008). India is one of the countries which contain a lot of traditional knowledge in terms of herbal medicines their effect against various diseases (Bisht and Badoni, 2009). The scientific evidence was found to be lacking due to the traditional information that was kept confidential by the village vaidyas (Vedavathy, 2001).

The specific therapeutic effect of herbal plants contains several bioactive compounds. The radical scavenging activity of the medicinal plants has thrown light on their investigation for scientific empowerment (Hazra *et al.*, 2010). Antioxidants are applied in the food industry as well as in the cosmetic industry as the functional ingredient to prevent oxidative damages (Elzaawely and Tawata, 2012). The bioactive compounds are of very much interest for the scientific

advancement through natural antioxidants (Jayaprakasha and Rao, 2000). Since application of antioxidants become broad in various areas it is necessary to develop different types of novel antioxidative agents. The plant source of natural antioxidants are more favoured due to their eco-friendly nature (Adisa *et al.*, 2011; Sivakumar and Panneerselvam, 2011a, b). However, there is no scientific proof justifying the traditional use of *O. cochinchinense* leaves in the treatment of "bone healing". The present investigation was made to study the biochemically active natural products, observed antioxidative activities in the different solvent extracts prepared from the leaves of *Ormocarpum cochinchinense*.

## MATERIALS AND METHODS

**Plant materials:** The leaves of *O. cochinchinense* collected from local area of Villupuram District, Tamil Nadu, India during October-December-2012.

**Preparation of plant extract:** In green leaves were dried powdered using with a mechanical grinder and stored in a jib lack cover. Leaf powder (1.25 kg) was refluxed with different solvents, Dimethyl sulfoxamide (DMSO), Ethyl acetate (EtoAc), ethanol (EtOH), methanol (MeOH) and chloroform (CHCl<sub>3</sub>) for five days. The total filtrate was concentrated to dryness, in hot air oven at 32°C to render five solvent extract for investigation.

**Chemicals:** All were purchased from SD fine chemical company Mumbai and all chemicals were of analytical grade.

**Phytochemical screening:** The phytochemical analysis of the (DMSO, EtoAc, EtOH, MeOH, CHCl<sub>3</sub>) of *O. cochinchinense* leaf was carried out by standard methods as described in Evans (2000), Harborne (1998) and Ghani (2003). Specifically, the extract was screened for the presence of secondary metabolites (flavonoids, saponins, glycosides, steroids, alkaloids, resins, tannins, terpenoids and acidic compounds and macronutrient carbohydrate, reducing sugar).

**Chemical group tests of the extract:** Testing different chemical groups present in the extract were performed the through phytochemical studies (Evans, 2000). In each test 10% (w/v) solution of extract was taken unless otherwise mentioned in individual test.

### Active-principle analysis

**Test for flavonoids:** Two hundred milligram each of the extract from different solvent was heated with 10 mL of ethyl acetate in boiling water for 3 min. The mixture was filtered and the filtrate was used for the following tests:

- **Ammonium test:** Four milliliter of the filtrate was shaken with 1 mL of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration at the ammonia layer indicated the presence of flavonoids
- **Ammonium chloride test:** Four milliliter of the filtrates was shaken with 1 mL of 1% aluminum chloride solution and observed for light yellow coloration. A yellow precipitate indicated the presence of flavonoids

**Test for glycosides:** Dilute sulphuric acid (5 mL) was added to 0.1 g of the test extract in a test tube and boiled for 15 min in a water bath. It was then cooled and neutralized with 20%

potassium hydroxide solution. A mixture, 10 mL of equal parts of Fehling's solution A and B were added and boiled for 5 min. A more dense red precipitate indicated the presence of glycoside.

**Test for steroids and terpenoids:** Nine milliliter of ethanol was added to extract and refluxed for a few minute and filtered. Each of the filtrates was concentrated to 2.5 mL in a boiling water bath. Five mL distilled water was added to the concentrated solution, the mixture was allowed to stand for 1 h and the waxy matter was filtered off. The filtrate was extracted with 2.5 mL of chloroform using a separating funnel. To 0.5 mL of the chloroform extract in a test tube, 1 mL of concentrated  $H_2SO_4$  was carefully added to form a lower layer. A reddish brown interface showed the presence of steroids.

A quantity, 0.5 mL of the chloroform extract was evaporated to dryness on a water bath and heated with 3 mL of concentrated  $H_2SO_4$  for 10 min on a water bath. A grey colour indicated the presence of terpenoids.

**Test for alkaloids:** A quantity (0.2 g) of the sample solvent was boiled with 5 mL of 2% HCl on steam bath. The mixture was filtered and 1 mL filtrate was treated with 2 drops of the following reagents:

- **Dragendorff's reagent:** A red precipitate was formed indicating presence of alkaloids
- **Wagner's reagent:** A reddish-brown precipitate was formed indicating presence of alkaloids
- **Hager's reagent:** A yellow precipitates was formed indicating the presence of alkaloids

**Test for saponins:** A quantity of 500 mg of the extract solvent was boiled with 5 mL of distilled water for 5 min. The mixture was filtered while still hot and the filtrate was used for the following test:

- **Frothing test:** A quantity, 1 mL of the filtrate was diluted with 4 mL distilled water. The mixture was shaken vigorously and then observed on standing for a stable froth

**Test for tannins:** A quantity, 2 g of the extract solvent was boiled with 5 mL of 45% ethanol for 5 min. The mixture was cooled and filtered. The filtrate was subjected to the following tests:

- **Lead-acetate test:** A 1 mL of the filtrate was added to 3 drops of the lead-acetate solution. A cream gelatinous precipitate indicates the presence of tannins
- **Ferric chloride test:** A quantity (1 mL) of the filtrate was diluted with distilled water and added 2 drops of ferric chloride. A transient greenish to black colour indicates the presence of tannins

**Test for acidic compounds:** A quantity, 0.1 g of the extract was placed in a clear dry test tube and sufficient water added. These were warmed differently in a hot water bath and cooled. A piece of water-wetted litmus paper was dipped into the filtrate and observed for colour change. Acidic compounds turn blue litmus paper red.

**Test for resins:** Two tests were carried out to detect the presence of resins in the plant part extract under investigation:

- **Precipitate test:** A quantity, 0.2 g of the extract was treated with 15 mL of 96% ethanol. The alcoholic extract was then poured into 20 mL of distilled water in a beaker. A precipitate occurring indicates the presence of resins
- **Colour test:** A quantity, 12 mg of the extract was treated with chloroform and extracts concentrated to dryness. The residues were re-dissolved in 3 mL of acetone and 3 mL of concentrated HCl was added. The mixture were now heated differently in a water bath for 30 min. Pink colour which changed to magenta red, indicated the presence of resins

**Macronutrients analyses:** The test which would be shown subsequently, were carried out to determine the presence of macronutrients in the herb *O. cochinchinense* leaf.

#### **Test for protein**

**Burette test:** A quantity, 2 mL of the extract was put in a test tube and 5 drops of 1% hydrated copper sulphate and 2 mL of 40% sodium hydroxide were added and the test-tube was shaken vigorously to mix the contents. A purple coloration showed the presence of proteins (presence of two or more peptide bonds).

**Test for carbohydrate:** A quantity, 0.1 g of the extract was shaken vigorously with water and then filtered. To the aqueous filtrate, few drops of molisch reagent were added, followed by concentrated H<sub>2</sub>SO<sub>4</sub> (1 mL) was carefully added to form a layer below the aqueous solution. A brown ring at the interface indicated the presence of carbohydrates.

**Test for reducing sugar:** A quantity, 0.1 g of the extract was shaken vigorously with 5 mL of distilled water and filtered. To the filtrate equal volumes of Fehling solution A and B were added and shaken vigorously. A brick red precipitate indicated the presence of reducing sugars.

#### ***In vitro* test for antioxidant activity**

**Free radical scavenging activity by DPPH method:** Quantitative Assay was performed on the basis of the modified method of Gupta *et al.* (2003). Stock solutions (10 mg mL<sup>-1</sup>) of the plant extracts were prepared in different solvent from which serial dilutions were carried out to obtain concentrations of 10, 20, 30, 40, 50, 100 and 500 mg mL<sup>-1</sup>. A quantity (2 mL) of diluted solutions were added to 2 mL of a 0.004% ethanol solution of DPPH, mixed and allowed to stand for 30 min for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibition were calculated. The experiment was performed in duplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control.

DPPH free radical scavenging activity was determined by the method described by Choi *et al.* (2007) and Desmarchelier *et al.* (1997). Plant extract (0.1 mL) was added to 3 mL of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min and percentage inhibition was calculated:

$$\frac{A_0 - A_1}{A_0} \times 100$$

where, A<sub>0</sub> is the absorbance of the control and A<sub>1</sub> is the absorbance of the extract/\_standard. IC<sub>50</sub> value was calculated from the equation.

**Statistical analysis:** Statistical analysis of the results was performed by student's t-test for independent samples. Values of  $p < 0.001$  were considered significant.

**RESULTS**

**Phytochemical and macronutrient screening:** The phytochemical screening of DMSO from the *O. cochinchinense* leaves indicated the presence of alkaloids, steroids, glycosides, saponins, tannins, acidic compounds, resins and coumerines but not flavanoids, acidic compounds, resins and coumerins. Quantitatively, alkaloids were more whereas resins were least among the detected secondary metabolites (Table 1). Furthermore, only carbohydrates (in moderate abundance) were found to be present in the DMSO of *O. cochinchinense* (Table 2).

Ethyl acetate (EtoAc) extract of *O. cochinchinense* leaves indicated the presence of flavanoids, steroids, glycosides, saponins, tannins, acidic compounds, resins and coumerines but not detected alkaloids. Quantitatively, flavanoids were more whereas resins were least among the detected secondary metabolites (Table 1). Furthermore, only carbohydrates (in moderate abundance) were found to be present in the ethyl acetate of *O. cochinchinense* (Table 2).

Ethanol extract of *O. cochinchinense* leaves indicated the presence of flavanoids, alkaloids, steroids, glycosides, saponins, tannins, acidic compounds, resins and coumerines but not fats and oils. Quantitatively, alkaloids were more whereas resins were least among the detected secondary metabolites (Table 1). Furthermore, only carbohydrates (in moderate abundance) were found to be present in the ethanol of *O. cochinchinense* (Table 2).

Phytochemical screening of methanol from the *O. cochinchinense* leaves indicates the presence of flavanoids, steroids, glycosides, saponins, tannins, acidic compounds, resins and coumerines but not detected alkaloids and acidic compounds. Quantitatively, flavanoids were more whereas resins were least among the detected secondary metabolites (Table 1). Furthermore, only carbohydrates (in moderate abundance) were found to be present in the methanol of *O. cochinchinense* (Table 2).

Table 1: Results of phytochemical analyses on *O. cochinchinense* leaves using the different solvent extracts

Tests for secondary metabolites	DMSO	EtoAc	EtOH	MeOH	CHCl <sub>3</sub>
Flavonoids	-	++	+++	++	-
Alkaloids	+	-	+++	++	++
Steroids	+	+++	++	++	+
Terpenoids	+	++	+++	+	+
Glycosides	++	++	+++	+	+
Saponins	+	++	+++	++	+
Tannins	+	+	+++	+	+
Acidic compounds	-	+	++	-	-
Resins	-	+	++	+	-
Coumarins	-	+	++	++	-
Fats and oils	Nd	Nd	Nd	Nd	Nd

-: Absent, +: Low in abundance, ++: Moderate in abundance, +++: High in abundance and Nd: Not detected

Table 2: Results of macronutrient analyses on *O. cochinchinense* leaves using the test solvent extracts

Tests for macronutrients	DMSO	EtoAc	EtOH	MeOH	CHCl <sub>3</sub>
Protein	Nd	Nd	Nd	Nd	Nd
Carbohydrates	++	++	+++	++	+
Reducing sugar	+	+	+++	++	-

-: Absent, +: Low in abundance, ++: Moderate in abundance, +++: High in abundance and Nd: Not detected

Chloroform extract of *O. cochichinense* leaves indicates the presence of alkaloids, steroids, glycosides, saponins, tannins, acidic compounds, but not detected resins, coumerines fats and oils. Quantitatively, alkaloids were more whereas resins were least among the detected secondary metabolites (Table 1). Furthermore, only carbohydrates (in moderate abundance) were found to be present in the ethanol of *O. cochichinense* (Table 2).

**In vitro antioxidant activity**

**DPPH free radical scavenging activity:** Dimethyl sulfoxamide extract of *O. cochichinense* showed potential antioxidant activity where the IC<sub>50</sub> was 13.05±0.39 µg mL<sup>-1</sup> (p<0.001), as compared that of ascorbic acid (IC<sub>50</sub> 6.10±0.18 µg mL<sup>-1</sup>) (p<0.001) which is well known antioxidant (Table 3). The extract caused an increase in DPPH free radical scavenging activity (% inhibition) as increasing dose (Table 4). This table showed most potent inhibitor with a (IC<sub>50</sub> 83.36±0.43 µg mL<sup>-1</sup>) which was comparable to ascorbic acid (IC<sub>50</sub> 85.12±1.25 µg mL<sup>-1</sup>).

Ethyl acetate (EtoAc) extract of *O. cochichinense* showed potential antioxidant activity where the IC<sub>50</sub> was 14.08±0.42 µg mL<sup>-1</sup> (p<0.001), as compared that of ascorbic acid (IC<sub>50</sub> 7.18±0.21 µg mL<sup>-1</sup>) (p<0.001) which is well known antioxidant (Table 3). Ethyl acetate extract caused an increase in DPPH free radical scavenging activity (% inhibition) as increasing dose (Table 4). EtoAc extract most potent the hydroxyl radical inhibition tests (IC<sub>50</sub> 83.33±0.40 µg mL<sup>-1</sup>) which was comparable to (IC<sub>50</sub> 84.68±1.20 µg mL<sup>-1</sup>) ascorbic acid.

Ethanol extract of *O. cochichinense* showed potential antioxidant activity where the IC<sub>50</sub> was 15.02±0.45 µg mL<sup>-1</sup> (p<0.001), as compared that of ascorbic acid (IC<sub>50</sub> 8.15±0.24 µg mL<sup>-1</sup>) (p<0.001) which is well known antioxidant (Table 3). The ethanol extract caused an increase in DPPH free radical scavenging activity (% inhibition) as increasing dose (Table 4). As the ethanol extract exhibited considerable free radical inhibition properties are (IC<sub>50</sub> 82.89±0.41 µg mL<sup>-1</sup>) which was

Table 3: DPPH free radical scavenging activity of *O. cochichinense* leaves using the different solvent extract

Sample	IC <sub>50</sub> (µg mL <sup>-1</sup> )	Standard	IC <sub>50</sub> (µg mL <sup>-1</sup> )
DMSO of <i>O. cochichinense</i>	13.05±0.39*	Ascorbic acid	06.10±0.18*
EtoAc of <i>O. cochichinense</i>	14.08±0.42*	Ascorbic acid	07.18±0.21*
EtOH of <i>O. cochichinense</i>	15.02±0.45*	Ascorbic acid	08.15±0.24*
MeOH of <i>O. cochichinense</i>	16.12±0.48*	Ascorbic acid	09.10±0.27*
CHCl <sub>3</sub> of <i>O. cochichinense</i>	14.88±0.44*	Ascorbic acid	08.18±0.23*

Table 4: DPPH radical scavenging activity of *O. cochichinense* leaves using the different solvent extract

Concentration of DMSO, Ascorbic acid, EtoAc, EtOH (µg mL <sup>-1</sup> )	Sample-DMSO	Ascorbic acid	Sample-EtoAc	Ascorbic acid	Sample-EtOH	Ascorbic acid
10	3.26±0.19	9.56±0.90	3.02±0.18	9.12±0.89	3.16±0.18	9.06±0.89
20	15.50±0.10	28.69±1.19	16.41±0.11	29.69±1.19	16.46±0.14	30.69±1.19
30	56.88±0.80	78.79±0.72	57.67±0.82	79.65±0.80	58.99±0.87	81.86±0.81
40	80.84±1.10	82.87±0.92	82.22±1.10	83.78±1.01	81.33±1.10	82.75±1.04
50	83.36±0.43	85.12±1.25	83.33±0.40	84.68±1.20	82.89±0.41	84.18±1.31
100	84.63±0.80	86.14±1.38	85.74±0.84	86.54±1.39	84.73±0.83	86.16±0.84
200	84.40±0.20	86.17±1.02	85.64±0.22	87.81±1.02	86.44±0.24	87.98±1.03
300	86.58±0.29	88.14±1.08	87.74±0.28	88.91±1.06	86.45±0.27	87.78±1.04
400	87.60±0.31	89.10±0.48	88.14±0.36	89.02±0.49	87.44±0.29	89.88±0.54
500	87.60±0.35	91.10±0.58	89.14±0.40	90.02±0.56	88.48±0.33	91.14±0.56

Values represent the Mean±SED, No. of readings in each group = 3

Table 5: DPPH radical scavenging activity of *O. cochinchinense* leaves using the different solvent extract

Concentration of MeOH, Ascorbic acid, CHCl <sub>3</sub> (µg mL <sup>-1</sup> )	Sample-MeOH	Ascorbic acid	Sample-CHCl <sub>3</sub>	Ascorbic acid
10	3.12±0.18	9.04±0.88	3.22±0.16	9.17±0.89
20	15.97±0.10	29.69±1.20	14.41±0.12	28.89±1.29
30	57.67±0.80	80.68±0.81	58.137±0.86	75.65±0.78
40	82.74±1.10	84.75±1.13	81.21±1.11	83.88±1.07
50	84.73±0.40	85.76±1.32	83.13±0.42	85.68±1.25
100	85.34±0.79	86.17±1.38	85.04±0.83	87.54±1.37
200	85.50±0.24	87.82±1.02	85.88±0.28	87.88±1.32
300	86.49±0.27	88.18±1.03	87.77±0.30	89.41±1.09
400	88.49±0.31	89.18±0.48	88.25±0.36	90.02±0.50
500	89.60±0.35	91.38±0.58	89.34±0.40	92.12±0.56

Values represent the Mean±SED, No. of readings in each group = 3

comparable to (IC<sub>50</sub> 84.18±1.31 µg mL<sup>-1</sup>) ascorbic acid. Table 4 and 5 indicated that hydroxyl radical was scavenged from ethanol extract in a dose dependent manner. The other solvent extracts appeared to have relatively higher or lower activities.

Methanol extract of *O. cochinchinense* showed potential antioxidant activity where the IC<sub>50</sub> was 16.12±0.48 µg mL<sup>-1</sup> (p<0.001), as compared that of ascorbic acid (IC<sub>50</sub> 9.10±0.27 µg mL<sup>-1</sup>) (p<0.001) which is well known antioxidant (Table 3). Methanol extract caused an increase in DPPH free radical scavenging activity (% inhibition) as increasing dose (Table 5). This table showed most potent the hydroxyl radical scavenge inhibition test (IC<sub>50</sub> 84.73±0.40 µg mL<sup>-1</sup>) which was comparable to (IC<sub>50</sub> 85.76±1.32 µg mL<sup>-1</sup>) ascorbic acid.

Chloroform extract of *O. cochinchinense* showed potential antioxidant activity where the IC<sub>50</sub> was 14.88±0.44g mL<sup>-1</sup> (p<0.001), as compared that of ascorbic acid (IC<sub>50</sub> 8.18±0.23 µg mL<sup>-1</sup>) (p<0.001) which is well known antioxidant (Table 3). The extract caused an increase in DPPH free radical scavenging activity (% inhibition) as increasing dose (Table 5). The CHCl<sub>3</sub> extract are free radical inhibition tests (IC<sub>50</sub> 83.13±0.42 µg mL<sup>-1</sup>) which was comparable to (IC<sub>50</sub> 85.68±1.25 µg mL<sup>-1</sup>) ascorbic acid.

## DISCUSSION

The preliminary phytochemical screening of the chemical constituents of different solvents from the *O. cochinchinense* plants showed that the leaves generally contain the major secondary metabolites in moderate abundance. In higher plants the flavonoids becomes inseparable with the antioxidant potentials that could cure heart diseases and also cancer (Noroozi *et al.*, 1998; Al-Humaid *et al.*, 2010). Vitamin A, C and E and flavonoids from plant sources are antioxidants in diet (Pietta, 2000). Thus, the absence of flavonoids in the DMSO extract of *O. cochinchinense* leaves might limit the solvent choice of DMSO in the extraction of active medicinal ingredients from *O. cochinchinense* leaves. Hence, the moderate abundance of alkaloids in the leaves of *O. cochinchinense* appears to support the efficacy of the use of the leaves in ethno-medicinal practice. The absence of alkaloids in the (EtoAc) extract of *O. cochinchinense* leave limit the solvent choice of (EtoAc) in the extraction of active medicinal ingredients from *O. cochinchinense* leaves.

The presence of flavonoids from ethanol extracts of *O. cochinchinense* leaves observed in the present study also attests to the possible efficacy of therapeutic use of *O. cochinchinense* leaves. They are related to sex hormones and could, by serving as potent starting material in the



synthesis of sex hormones, ensure such hormonal balance (Okwu, 2001). This could in addition of the noted high carbohydrate content of *O. cochinchinense* leaves that could provide useful energy, be highlighting the possible reproductive benefits from the prospective use of *O. cochinchinense* leave as nutraceutical beverage. The absence of acidic compounds from methanol extract of *O. cochinchinense* leaves might limit the solvent choice of MeOH in the extraction of active medicinal ingredients from *O. cochinchinense* leaves. The absence of flavonoids, acidic compounds, resins, coumerins in the CHCl<sub>3</sub> extract of *O. cochinchinense* leaves might limit the CHCl<sub>3</sub> solvent choice of extraction and active medicinal ingredients from *O. cochinchinense* leaves. The phytochemical analysis of the extract showed the presence of flavonoids, alkaloids, saponins, sterols, terpenoids, resins and sugar. These constituents may be responsible for antioxidant potentiality of *O. cochinchinense*.

## CONCLUSION

The present study reveals that different solvent extracts of *O. cochinchinense* leaves possesses significant of phytochemical screening and antioxidative activity. Now research is continued to isolate lead compound from these extracts and also to develop a potent formulation for treatment of bone healing activities.

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