

IN SITU BIO-ACTIVATION AND INACTIVATION MECHANISM IN *PSORALEA CORYLIFOLIA* EXTRACT AND ASSOCIATED SAFETY FOR PSORIASIS AND VITILIGO

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Abstract

The present study deals with the in situ bio-activation and inactivation mechanism of *Psoralea corylifolia* extract and its associated safety. The natural extracts of *Psoralea corylifolia* showed photosensitivity only instantaneously whereas after 30 minutes no photosensitivity was observed. The possible dermal reaction with various other bio-molecules of *Psoralea corylifolia* extract may be 'switching off' the activity of Trioxsalen and Methoxsalen. The programmed inactivation of photosensitivity of *Psoralea corylifolia* extract clearly highlights its efficacy and safety over synthetic derivatives of Psoralen- Trioxsalen and Methoxsalen. TLC, Spectrogram, cyanotype paper based photo reaction and volunteer based study were conducted to establish the above scientific fact. Details are presented in the paper.

Keywords:

Psoralen, Psoralea corylifolia, Trioxsalen, Methoxsalen, Vitiligo, Psoriasis, Furocoumarins.

Introduction

Psoralen derivatives such as Trioxsalen, Methoxsalen from *Psoralea corylifolia* and its usefulness in the treatment of Vitiligo and Psoriasis is well known. The natural Psoralen is otherwise called as Furocoumarins, or furanocoumarins are a group of organic chemical substances known to be present in wide variety of plants. Psoralen is known to induce photosensitivity in human skin and therefore it has enormous medical value for the treatment of various skin diseases [1,2,3,4].

Synthetic Psoralen has gained significant importance in photo therapy for Psoriasis and Vitiligo. The oral administration of synthetic Psoralen is known to produce certain unpleasant side effects and hence its use is highly limited. The natural counter part of the synthetic Psoralen, the furanocoumarins (Trioxsalen or Methoxsalen) if prepared topically can replace the oral synthetic Psoralen and also the associated side effects can be avoided [5,6,7,8]. However, the stigma of photo-toxicity and the associated side effects around the synthetic Psoralen forbid dermatologist from accepting even the natural furanocoumarins based preparations which are relatively safe.

We, in the present paper discuss the 'short-life of Trioxsalen and Methoxsalen' in native form' isolated from *Psoralea corylifolia* on human skin (topical application) and how the 'short-life' may enable better treatment scope for Psoriasis and Vitiligo without any side effects.

We hypothesize that the faster metabolism of Trioxsalen and Methoxsalen at the dermal level or the possible modification of Trioxsalen and Methoxsalen due to other biomolecules present in the extract or the photo- protection offered by the 'interacted compound (s)' may be the reason for the short lived photosensitivity.

This is the first study that establishes the 'short-life' of natural Psoralen- Trioxsalen and Methoxsalen. The possible in situ 'bio-activation' and 'bio-inactivation' mechanism of Trioxsalen and Methoxsalen in the natural extract of *Psoralea corylifolia* and associated safety are discussed in the paper.

Materials and methods

Extraction *Psoralea corylifolia*

The shade dried seeds of *Psoralea corylifolia* were ground to powder and then treated with hydro alcohol at 1:1 ratio. The proportion of seeds used for the extraction was 10%. The resultant extract was filtered, and then distilled to separate the alcohol. The pasty extract thus obtained from *Psoralea corylifolia* seeds were used for further study. Similarly various other solvents such as methanol, chloroform, ethyl acetate, pet ether and hydro-alcohol were also used for extraction and the proportion of seeds used was 10% as described above.

TLC separation

The extracts of *Psoralea corylifolia* obtained with various solvents and standard Methoxsalen and Trioxsalen were studied by thin layer chromatography using benzene: chloroform: ethyl acetate (8:1:1) as mobile phase as well as toluene: ethyl acetate (7.5:2.5). The TLC plates were observed under UV & visible light.

Spectroscopic study

The extract of *Psoralea corylifolia* obtained with various solvents and the standards Methoxsalen and Trioxsalen were studied by spectrophotometry at the wavelength ranged between 200 to 800 nm

Evaluation of Photosensitivity in cyanotype paper

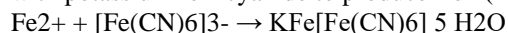
The extracts of *Psoralea corylifolia* obtained with various solvents and the standards Methoxsalen and Trioxsalen were studied for photosensitivity using cyanotype paper. The cyanotype paper was prepared and the photo reaction was measured by adopting the method described by Aruna et al., 2017. The stability of various extracts in inducing photosensitivity and Methoxsalen, Trioxsalen in cyanotype paper at different time intervals was also studied [9].

Principle and method of preparation

The principle behind the use of cyanotype paper is the photo conversion of ferric ion to ferrous ion which in turn reacts with potassium ferric cyanide. The use of blueprints or cyanotypes dates back to the 1840's when Sir John Herschel discovered the light sensitivity of certain iron salts.

In brief, to make a cyanotype paper, the paper is treated with a solution of potassium ferricyanide ($K_3Fe(CN)_6$) and ferric ammonium citrate ($Fe(NH_4)$) and shade dried to fix the chemicals.

When paper is exposed to light, the light reduces the ferric ion (+3) to ferrous ion (+2). The ferrous ions then react with potassium ferricyanide to produce iron (III) hexacyanoferrate (II) by the following reaction:



Iron (III) hexacyanoferrate (II), an insoluble, deep-blue colored compound called Prussian blue, forms the blue part of the final blueprint. Prussian blue can exist with different amounts of cations and water in the crystal lattice, so the exact molecular formula can vary depending upon conditions. To fix the image, the paper is washed with water to remove the unreacted water-soluble salts, leaving the areas not exposed to light as white areas on the blueprint.

The entire experiment of handling the paper, application of the test materials, etc., were done in dark chamber until the paper is exposed to sun.

All the above papers except the untreated control in quadruplet were exposed to sun simultaneously for 5 min. After 5 min exposure the papers were brought back to dark chamber for a period of rest for 5 min, washed with distilled water and then examined the colour change.

The paper that that turn blue indicate photo reaction that paper remain white show no photo reaction.

Photosensitivity of *Psoralea corylifolia* extracts in human skin vis-à-vis time

5 volunteers each were used for the study. 2cm² areas were demarcated in the volar skin and eight separate areas were marked. The extracts of *Psoralea corylifolia* in following solvents such as hydro-alcohol, ethanol, methanol, chloroform, ethyl acetate, pet ether were used for the study. Similarly, Trioxsalen and Methoxsalen were also studied.

After drying out the solvents the residual extracts at 2mg was applied in the pre-identified demarcated 2cm² area and then the forearm region was selectively exposed to sun for 10 minutes. Similarly, the above procedure was repeated in the above volunteers in the untreated volar forearm but the sun exposure was done after 20 minutes of application of the sample. The extent of erythema formation in the test sites versus untreated sites was observed and recorded. Similarly, the difference in the erythema formation immediately after sample application and after 20 minutes hold up time was also recorded.

Results**TLC separation**

Although the extract of *Psoralea corylifolia* obtained with various solvents such as methanol (M), Ethanol (E), Hydro alcohol (H A), Chloroform (C), Ethyl acetate (EA), Pet ether (PE,) lane 1 to lane 6 showed slight variation in the profile but all the extracts showed the presence of Trioxsalen when the mobile phase of Benzene: chloroform: ethyl acetate (8:1:1). Lane 1 to 7 (Fig 1 & 1a)

However the presence of Methoxsalen could be established in the above extract only when the mobile phase of toluene: ethyl acetate (7.5: 2.5). Lane 1 to 6 and lane 8 (Fig- 2 & 2 a)

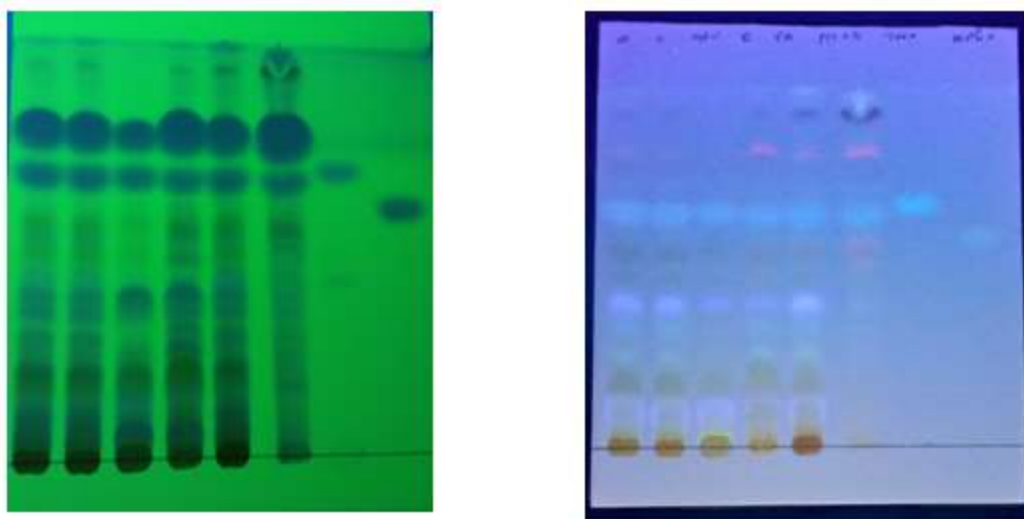


Fig 1 & 1 a- TLC separation (UV short wavelength 254 nm and UV long wavelength 366 nm)

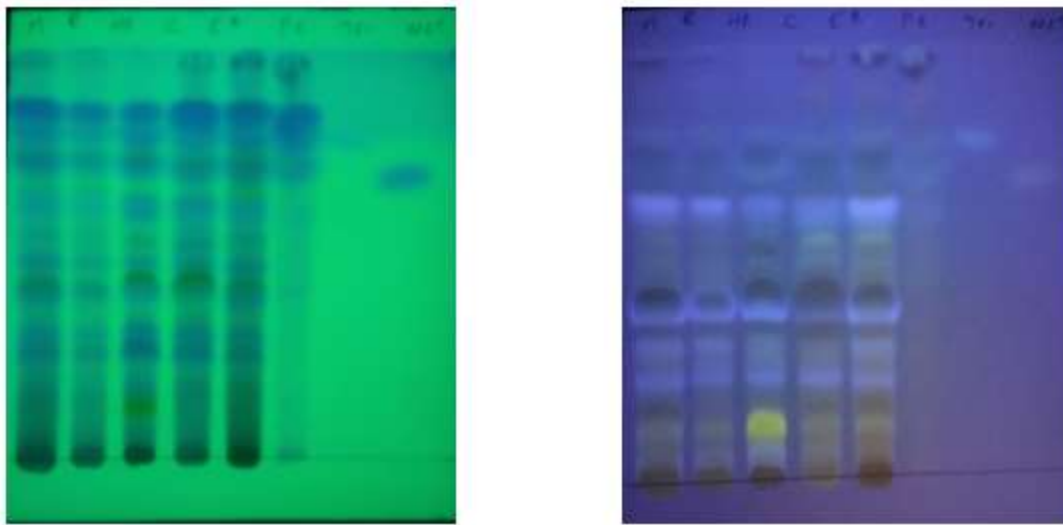
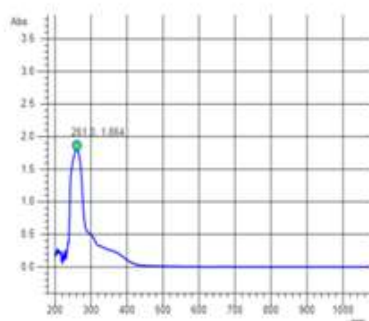


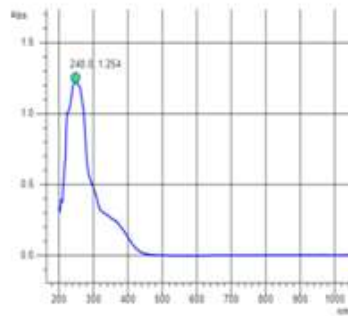
Fig 2 & 2 a TLC separation (UV short wavelength 254 nm and UV long wavelength 366 nm)

Spectrophotometry- UV spectrogram of *Psoralea corylifolia* extracted using different solvents

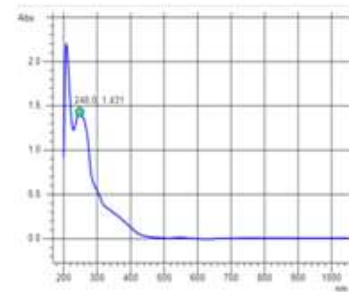
The spectrophotometric analysis reveals the presence of Methoxsalen in ethanol, methanol and pet ether extracts of *Psoralea corylifolia* at 248 nm. However, the other peak of Methoxsalen at 303 nm we could not find in any of the above extracts. Interestingly the peak values of Trioxsalen at 254 & 524 nm also we could not establish in any of the extracts of *Psoralea corylifolia*. Fig 3 to fig 8



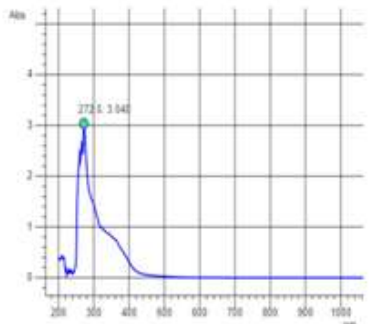
Chloroform extract



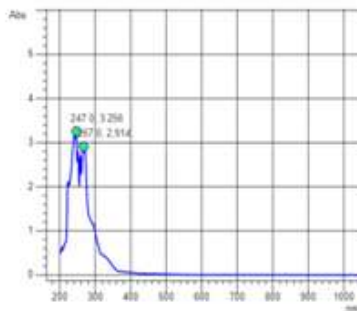
Ethanol extract



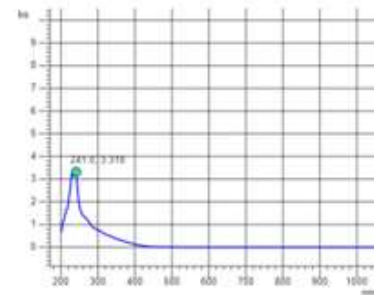
Methanol extract



Ethyl acetate extract

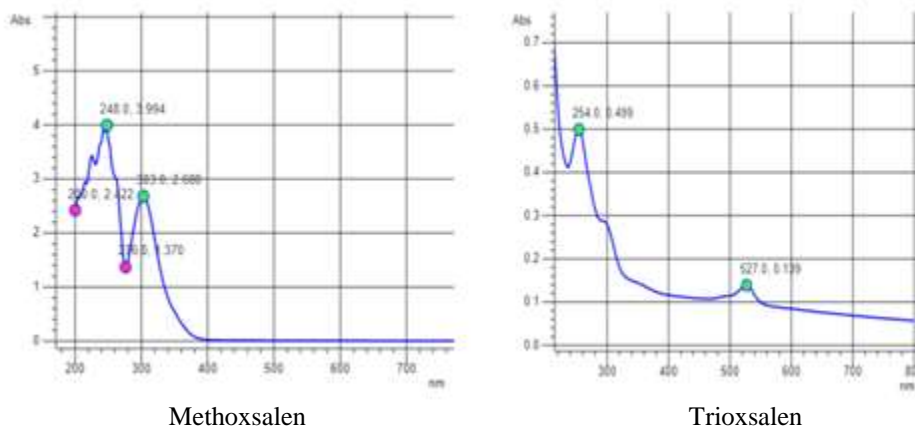


Pet ether extract



Hydro alcohol extract

Fig- 9 & 10 Standard UV spectrum of Methoxsalen and Trioxsalen



Evaluation of Photo-reaction in cyanotype paper

The untreated cyanotype paper turned white from yellow on sun exposure. The cyanotype paper treated with ethanol, methanol, chloroform, pet ether, ethyl acetate & hydro alcohol extracts of *Psoralea corylifolia* showed photo response during sun exposure and the paper turned to blue colour from yellow. Similarly, the cyanotype paper treated with Trioxsalen and Methoxsalen also turned to blue from yellow during sun exposure- Table 1.

Table- 1 Response of cyanotype paper to sun exposure

Treatment	Initial color of the paper	Colour change on sun exposure	
		White	Blue
Untreated paper kept in dark chamber	Yellow	+	-
Ethanol extract	Yellow	-	+
Methanol extract	Yellow	-	+
Chloroform extract	Yellow	-	+
Ethy acetate extract	Yellow	-	+
Pet ether extract	Yellow	-	+
Hydro alcohol extract	Yellow	-	+
Trioxsalen	Yellow	-	+
Methoxsalen	Yellow	-	+

Photo responsiveness of cyanotype paper vis-à-vis time

The cyanotype paper treated with the extract of *Psoralea corylifolia* in various solvents as well as Methoxsalen and Trioxsalen was studied at different time intervals to understand whether the extracts show activity. As soon as the cyanotype paper was prepared were kept in dark chamber until use. The extract treated cyanotype paper showed photo responsiveness even after 15 & 30 days of storage indicating no decay in the activity of the extracts. Similarly, Methoxsalen & Trioxsalen treated cyanotype paper after 15 and 30 days of storage also photo responsiveness during sun exposure. Table- 2

Table- 2 Photo responsiveness of cyanotype paper vis-à-vis time

Treatment	Age of the paper in days	Initial colour of the paper	Colour change on sun exposure	
			White	Blue
Untreated paper kept in dark chamber	15	Yellow	+	-
	30	Yellow	+	-
Ethanol extract	15	Yellow	-	+
	30	Yellow	-	+
Methanol extract	15	Yellow	-	+
	30	Yellow	-	+
Chloroform extract	15	Yellow	-	+
	30	Yellow	-	+
Ethyl acetate extract	15	Yellow	-	+
	30	Yellow	-	+
Pet ether extract	15	Yellow	-	+
	30	Yellow	-	+
Hydro alcohol extract	15	Yellow	-	+
	30	Yellow	-	+
Trioxsalen	15	Yellow	-	+
	30	Yellow	-	+
Methoxsalen	15	Yellow	-	+
	30	Yellow	-	+

Photosensitivity of *Psoralea corylifolia* extracts in human skin vis-à-vis time

The extent of erythema immediately after application of various extracts of *Psoralea corylifolia* was quite high. Similarly, the erythema formation was intense in Methoxsalen and Trioxsalen applied areas. Table- 3

S.no	Treatment	Extent of erythema
1	Untreated control not expose to sun	-
2	Untreated control	+
3	Ethanol extract	+++
4	Methanol extract	+++
5	Chloroform extract	+++
6	Ethy acetate extract	+++
7	Pet ether extract	+++
8	Hydro alcohol extract	+++
9	Trioxsalen	+++
10	Methoxsalen	+++

The extent of erythema in volar forearm after 20 minutes of application of various extracts of *Psoralea corylifolia* was minimal and was comparable to control site. Whereas the erythema formation in Trioxsalen and Methoxsalen applied region after 20 minutes was intense. Table- 4

S.no	Treatment	Extent of erythema
1	Untreated control not expose to sun	-
2	Untreated control	+
3	Ethanol extract	+
4	Methanol extract	+
5	Chloroform extract	+
6	Ethy acetate extract	+
7	Pet ether extract	+
8	Hydro alcohol extract	+
9	Trioxsalen	+++
10	Methoxsalen	+++

= No erythema, + = very mild erythema, +++ = high/intense erythema

Discussion

The present study has brought out a new scientific possibility that the natural extract of *Psoralea corylifolia* in 'native form' will not only pharmaceutically active but also the 'native form' may provide great safety margin from side effects. The usefulness of Psoralen and its derivatives especially Methoxsalen and Trioxsalen for the treatment of various skin diseases such as Psoriasis, Vitiligo, Eczema etc., are well known and are followed widely all over the world [10, 11].

The oral Psoralen medication is found to increase the photo sensitivity of the skin, damage the DNA and triggers the genetic memory of melanocytes to synthesize melanin and thereby the Psoralen derivatives forms an important treatment precursor for Psoriasis and Vitiligo. However, the oral administration is also known to cause several unpleasant side effects such as nausea, vomiting, cramps etc. Therefore, Psoralen derivatives are although useful; not widely sort after by clinicians due to the potential side effects [12].

Psoralen derivatives are synthesized for clinical use, the popular among are Methoxsalen and Trioxsalen. The synthetic molecules are believed to be less toxic. However natural extract of *Psoralea corylifolia* is given less importance for the treatment of Vitiligo and Psoriasis may be due to the complex metabolites present in the crude extract of *Psoralea corylifolia*. Similarly, the topical preparations of Psoralen derivatives are also given less importance due to the phototoxic effect. The topical preparation will certainly avoid various side effects of oral administration but the pre-existing stigma of toxicity associated with Psoralen preparations scuttle any adventure of topical Psoralen preparations for the treatment of Vitiligo and Psoriasis.

The photo toxicity and DNA damaging property of the synthetic derivatives of Psoralen even in topical form is obvious but natural extracts of *Psoralea corylifolia* may offer the desired benefit of photo toxicity but at the same time may also offer protection to photo sensitive skin is less known, which we have established in our study.

The extract of *Psoralea corylifolia* in various solvents such as ethanol, methanol, hydro- alcohol, chloroform, ethyl acetate, pet ether showed reliable and comparable TLC profile. Similarly, the TLC profile of above extracts showed bands quite reliable to the bands obtained for standard Trioxsalen and Methoxsalen. On the contrary the spectrogram showed the peaks for the extracts of *Psoralea corylifolia* in various solvents similar to that of standard Methoxsalen.

The contradiction in the TLC & spectrogram of the extracts of *Psoralea corylifolia* with Trioxsalen & Methoxsalen needs to be understood from the medical point of view than from chemistry.

The cyanotype experiment has revealed that the extract of *Psoralea corylifolia* in various solvent systems produced photo reaction in cyanotype paper during sun exposure and so were Methoxsalen and Trioxsalen. The cyanotype paper prepared with the extract retained the photo responsiveness even after 30 days of storage clearly suggests that the photo reaction of the extracts of *Psoralea corylifolia* is quite stable.

To establish the difference in the photosensitivity of the extracts of *Psoralea corylifolia* vis-à-vis synthetic Methoxsalen and Trioxsalen, a volunteer based study was conducted. The above extracts and Methoxsalen and Trioxsalen at 2mg/cm² were applied over skin and then expose to sun for 10 minutes and then the extent of erythema was recorded. The extract of *Psoralea corylifolia* in various solvent systems showed the effect more or less same and we observed uniform photo sensitive reaction in the skin where different extracts were applied.

To understand the long lastingness of the effect of *Psoralea corylifolia* in various solvent systems in inducing photosensitivity, we have applied various extracts of *Psoralea corylifolia* (2mg/cm²) in volar forearm and then sun exposure was given after 20 minutes. Interestingly none of the extracts of *Psoralea corylifolia* such as ethanol, methanol, hydro- alcohol, chloroform, ethyl acetate, pet ether induced photosensitivity whereas Trioxsalen and Methoxsalen did induce photosensitivity.

Although a proper scientific explanation for the above is difficult to offer but we strongly believe that *Psoralea corylifolia* extract in 'native form' may have in situ bio activation and inactivation mechanism. When the extract of *Psoralea corylifolia* was applied over the skin, we presume the furocoumarin derivatives may make the skin photosensitive instantaneously. The other bio molecules in the extract may either terminate the furocoumarin reaction or may be offering photo protection to the skin after their absorption into the skin. This may be the reason why the photo sensitivity was pronounced only when the skin was exposed to sun immediately after the application of the extract of *Psoralea corylifolia*, whereas after 20 minutes the close end reaction may be terminating the photosensitivity reaction and therefore the skin was protected off from photosensitivity.

Clinical trial of topical Trioxsalen and oral Methoxsalen along with UV exposure showed a uniform response for both the preparations but the side effects were high with oral Methoxsalen. When the synthetic derivatives of Psoralen, Methoxsalen or Trioxsalen were used either as topical or oral administration, the side effects were imminent. Further the topical use of Trioxsalen may make the skin photosensitive on cumulative basis which is quite risky. Whereas the natural extract of *Psoralea corylifolia* may offer photo-induction instantaneously but within minutes, such reaction may disappear and thereby the chance of cumulative photosensitivity and photo-vulnerability of skin would reduce to minimal with natural extracts of *Psoralea corylifolia*.

The natural extracts of *Psoralea corylifolia* is a combination of various bio-active molecules and are likely to exhibit the therapeutic benefits individually and or collectively. Possibly one such individual benefit may be photosensitivity and the collective benefit may be skin protection and termination of photosensitivity within short span of time.

Although our findings may warrant further studies but our study has thrown sufficient scientific evidence for both the therapeutic benefits and safety of natural extracts of *Psoralea corylifolia* for various skin diseases such as Psoriasis and Vitiligo.

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