Standardization of Sitopaladi Churna: A Poly-Herbal Formulation

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ABSTRACT

Standardization of herbal formulation is essential in order to assess the quality of drugs for therapeutic value. According to an estimate of World Health Organization (W.H.O) nearly 80% of populations of developing countries rely on traditional medicines. The World Health Organization (WHO) in 1999 has given a detailed protocol for the standardization of herbal drugs comprising of a single content, but very little literature is available for the standardization of poly-herbal formulation. We have developed a simple scheme for standardization and authentication of sitopaladi Churna. Four marketed preparations and in-house preparations were used for the study, performed. The various parameters including organoleptic characteristics and physicochemical parameters were used. HPTLC was carried out for quantitative analysis of all the formulations. The set parameters were found to be sufficient to standardize the Sitopaladi Churna and can be used as reference standards for the quality control/quality assurance study mostly on plant drugs for their primary health care needs.

Keywords: Standardization; Sitopaladi Churna; Traditional medicine; Physicochemical parameters; HPTLC.

INTRODUCTION

India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Traditional health care has been flourishing in this country for many centuries. Ayurveda and other Indian systems of medicines may be explored with the modern scientific approaches for better leads in the health care [1]. In the last few decades, there has been an exponential growth in the field of ayurvedic medicine. There are great need of standardization and quality control of ayurvedic formulations. Standardization and quality control depends upon the nature of crude drug and compound drugs, it’s source i.e. factors associated with raw materials which are beyond of human control like seasonal, geographical, age...
of the plant, time of collection, type of drying etc. due to these natural conditions. the percentage of chemical constituents [2,3] of the drug does not remain uniform as our expectation. The need of quality control for ayurvedic drug is due to the fact that the preparation of drug according to the ancient method has been reduced due to the commercialization of ayurvedic pharmacy. The absence of post-market surveillance and the paucity of test laboratory facilities also make the quality control of aurvedic medicines exceedingly difficult at this time.[4] Therefore, an attempt has been made to standardize sitopaladi Churna, an Ayurvedic formulation as prescribed in Ayurvedic Formulary, used as anti-tussive, common cold etc. The individual plant powders of the formulation were subjected to various pharmacognostiical parameters. Three formulation, one in-house preparation and two samples from different manufactures were procured and subjected to various physicochemical analysis, TLC and HPTLC fingerprinting and botanical characterization using authenticate ingredients as controls.

MATERIALS AND METHODS

All the chemicals used in the experiment were of analytical grade. Menthol, n-hexane, Benzene, Toluene, Diethyl ether, α-terpinyl acetate, Piperine, & Cinnamaldehyde were purchased from Sigma Aldrich, USA. All the solvents used in the experiment were procured from Merck Specialities Pvt. Ltd, Mumbai, India.

Instruments

Spotting device: Linomat IV automatic sample spotter; CAMAG (Muttenz, Switzerland)
Syringe: 100µL Hamilton (Bonadug, Switzerland)
TLC chamber: Glass twin trough chamber (20×10×4).
Densitometer: TLC scanner 3 with CATS software; CAMAG
HPTLC Plate: 20×10cm, 0.2 precoted with silica gel 60F; Merck
pH meter: Elico Ltd., Hyderabad, India.
Muffle furnace: Dolphin Industries Ltd., Mumbai, Leica microscope (EZ-4D)

Plant material

Sitopaladi churna consist of like Sitopal(Cane sugar), Vamsolochana(vamsa), Pippalī(Piper longum fruit), Elā(Elettaria cardamomum seed), Tvak(Cinnamomum zeylanicum stem bark) All these ingredients were procured from the local Raw traders of Jeypore, Koraput, Odissa, India and all the plant material were authenticated by Mr. S.R. Dash H.O.D Dept of Botany Vikram Dev College Jeypore, Koraput Odisa. Voucher specimens (JCP/09/LAB-5/35) of the same have been deposited in the museum of Dept. of Pharmacognosy, Jeypore College of Pharmaceutical Sciences for future reference.

Preparation of sitopaladi churna

In house formulation of sitopaladi churna was prepared as per Ayurvedic Formulary of India. Take all the ingredients like Sitopal(Cane sugar) 192g., Vamsolochana(vamsa) 96g, Pippalī(Piper longum) 48g, Elā(Elettaria cardamomum) 24g & Tvak(Cinnamomum zeylanicum) 12g. Powder separately ingredients and pass through sieve number 80#. Weigh separately each powdered ingredient and mix together. Pass the Cūrna through sieve number 44 # to prepare a homogeneous blend. Pack it in tightly closed containers to protect from light and moisture.
Marketed samples
The marketed samples of various brands sitopaladi Churna i.e. Zandu (B), Baidyanath(C) and Dabur (A) and the in-house preparation(D) were standardized based on their organoleptic characters, physical characteristics and physicochemical properties.

Organoleptic Evaluation
Organoleptic evaluation refers to evaluation of formulation by color, odor, taste, texture etc. The organoleptic characters of the samples were carried out based on the method described by Siddique et. Al [5].

Table 1: Organoleptic properties of different Sitopaladi Churna formulation

<table>
<thead>
<tr>
<th>Different Formulation</th>
<th>Appearance</th>
<th>Color</th>
<th>Taste</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation A</td>
<td>powder</td>
<td>Whitish brown</td>
<td>Sweetish sweet</td>
<td>Fragrant</td>
</tr>
<tr>
<td>Formulation B</td>
<td>powder</td>
<td>Whitish brown</td>
<td>Sweetish sweet</td>
<td>Fragrant</td>
</tr>
<tr>
<td>Formulation C</td>
<td>powder</td>
<td>Whitish brown</td>
<td>Sweetish sweet</td>
<td>Fragrant</td>
</tr>
<tr>
<td>Formulation D</td>
<td>powder</td>
<td>Whitish brown</td>
<td>Sweetish sweet</td>
<td>Fragrant</td>
</tr>
</tbody>
</table>

*Samples are collected and labeled as Dabur, Zandu, Baidyanath, In house. Named as formulation A, formulation B, formulation C, formulation D respectively.

Physicochemical Investigation
Determination of total ash
Total ash determination constitutes detecting the physiological ash (ash derived from plant(tissue) and nonphysiological ash (ash from extraneous matter, especially sand and soil adhering to the surface of the drug). For its detection, 2g of powdered material of each formulation and the individual ingredients of the powers were placed separately in a suitable tared crucible of silica previously ignited and weighed. The powdered drugs were spread into an even layer and weighed accurately. The materials were incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash.[6]

Acid insoluble ash
The ash obtained as above was boiled for 5 min with 25ml of dilute hydrochloric acid; the insoluble matter was collected on an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash with reference to the air-dried drug was calculated.

Water soluble ash
The ash was boiled for 5 minutes with 25 ml of water; collected insoluble matter in an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash; the difference
in weight represents the water-soluble ash. The percentage of water-soluble ash with reference to the air-dried drug was calculated.

Table 2: Quality tests for different Sitopaladi Churna formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Alcohol Soluble (%)</th>
<th>Water Soluble (%)</th>
<th>Total ash (%)</th>
<th>Acid insoluble (%)</th>
<th>Water soluble (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamomum</td>
<td>47.3±0.93</td>
<td>17.8±0.06</td>
<td>4.8±0.54</td>
<td>3.6±0.31</td>
<td>7.2±0.46</td>
</tr>
<tr>
<td>Zeylanicum</td>
<td>3.48±0.02</td>
<td>24.84±0.28</td>
<td>4.2±0.28</td>
<td>1.4±0.247</td>
<td>8.3±0.33</td>
</tr>
<tr>
<td>Elettaria</td>
<td>9.29±0.02</td>
<td>15.32±0.67</td>
<td>3.9±0.63</td>
<td>1.9±0.215</td>
<td>9.2±0.02</td>
</tr>
<tr>
<td>Cordamomum</td>
<td>33.23±0.15</td>
<td>64.35±0.75</td>
<td>22.8±0.08</td>
<td>16.9±0.07</td>
<td>20±0.31</td>
</tr>
<tr>
<td>Formulation A</td>
<td>58.27±1.51</td>
<td>58.23±0.83</td>
<td>17.4±0.06</td>
<td>20.6±0.31</td>
<td>20.1±0.42</td>
</tr>
<tr>
<td>Formulation B</td>
<td>29.31±0.27</td>
<td>59.01±0.87</td>
<td>18.2±0.07</td>
<td>11.0±0.04</td>
<td>25±0.62</td>
</tr>
<tr>
<td>Formulation C</td>
<td>30.36±1.21</td>
<td>62.45±0.72</td>
<td>19.2±0.09</td>
<td>22.3±0.41</td>
<td>19.3±0.62</td>
</tr>
</tbody>
</table>

*Samples are collected and labeled as Dabur, Zandu, Baidyanath, Inhouse. Named as formulation A, formulation B, formulation C, formulation D respectively Determination of solvent Extractive values

Alcohol soluble extractive value

5g of coarsely powdered air-dried drug was macerated with 100ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug and is represented as % value.[6]

Water soluble extractive value

5g of coarsely powdered air-dried drug was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish at 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug and is represented as % value.[6]

Loss on drying

Loss on drying is the loss of mass expressed as percent w/w. About 10g of drug samples of each formulation was accurately weighed in a dried and tared flat weighing bottle and dried at 105°C.
for 5 hrs. Percentage was calculated with reference to initial weight.[6]

**Determination of pH**
The pH of different formulations in 1% w/V and 10% w/V of water soluble portions was determined using standard glass electrode at 240 according to the prescribed standard method in Indian Pharmacopoeia.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Loss on drying at 105°C</th>
<th>PH 10% w/v solution</th>
<th>PH 1% w/v solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation A</td>
<td>1.63±0.0226</td>
<td>7.3</td>
<td>7.1</td>
</tr>
<tr>
<td>Formulation B</td>
<td>2.77±0.0321</td>
<td>7.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Formulation C</td>
<td>2.14±0.0021</td>
<td>7.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Formulation D</td>
<td>3.17±0.0025</td>
<td>7.4</td>
<td>7.2</td>
</tr>
</tbody>
</table>

*Samples are collected and labeled as Dabur, Zandu, Baidyanath, Inhouse. Named as formulation A, formulation B, formulation C, formulation D respectively.

**Table 4: Powder fluorescence test of different formulation of sSitopaladi Churna**

<table>
<thead>
<tr>
<th>Material</th>
<th>D</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
<td>Day U.V</td>
<td>UV Day</td>
<td>U.V Day</td>
</tr>
<tr>
<td>Light</td>
<td>254 366</td>
<td>Light 254 366</td>
<td>Light 254 366</td>
</tr>
<tr>
<td>nm nm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P=50%H2SO4</td>
<td>Y.B L.G</td>
<td>L.G Y.B</td>
<td>L.G L.G</td>
</tr>
<tr>
<td></td>
<td>L.G</td>
<td>L.G Y.B</td>
<td>L.Y L.G</td>
</tr>
<tr>
<td>P=ConcHNO3</td>
<td>Y.B L.G</td>
<td>L.G Y.B</td>
<td>R.B L.G</td>
</tr>
<tr>
<td></td>
<td>L.G</td>
<td>L.G Y.B</td>
<td>L.B L.G</td>
</tr>
<tr>
<td>P=ConcH2SO4</td>
<td>R.B L.G</td>
<td>R.B L.G</td>
<td>Y.B Y.B</td>
</tr>
<tr>
<td>P=50%HNO3</td>
<td>Y.B G.Y</td>
<td>L.G L.Y</td>
<td>G.Y L.G</td>
</tr>
<tr>
<td>P=50%KOH</td>
<td>Y.B L.G</td>
<td>B L.G</td>
<td>L.G D.G</td>
</tr>
<tr>
<td>P-Iodine</td>
<td>Y.B B</td>
<td>B Y.B</td>
<td>G.Y B.G</td>
</tr>
<tr>
<td>IN HCl</td>
<td>L.Y L.G</td>
<td>L.G L.Y</td>
<td>L.G L.G</td>
</tr>
<tr>
<td>IN NaOH</td>
<td>Y.B B</td>
<td>L.G R.B</td>
<td>L.G L.G</td>
</tr>
</tbody>
</table>

*B-Black, Y.B-Yellowish black, L.G-Light green, L.Y-Light yellow, R.B-Brown, R.B-Reddish brown, D.B-Dull black, G.Y-Greyish yellow, D.G-Dark green*

**Fluorescence analysis**
One mg of powdered drugs of each formulation were exposed to ultraviolet light at wavelength of 254nm and 366nm and in daylight while wet after being treated with different reagents. Determination of physical Characteristics [7,8]
Bulk density and Tap density
The term bulk density refers to a measure used to describe a packing of particles or granules. The equation for determining bulk density \( D_b \), \( D_b = \frac{M}{V_b} \), where \( M \) is the mass of the particles and \( V_b \) is the total volume of the packing. The volume of the packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device (Jolting Volumeter) that has a specially cut rotating can. 100gm of weighed formulation powder was taken and carefully added to the cylinder with the aid of a funnel. Typically the initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the Bulk density value and after tapping the volume reduced, giving the value of tapped density.[7,8]

Angle of repose
Angle of Repose has been used as an indirect method of quantifying powder flowability because of its relationship with interparticle cohesion. As a general guide, powders with angle of repose greater than 50 degree have unsatisfactory flow properties, whereas minimal angle close to 25 degrees correspond to very good flow properties. The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height, which was taken 2.5 cm (H), above the graph paper that is place on flat horizontal surface. Powder or granulation was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. [7,8] \[ \tan \theta = \frac{H}{R} \text{ or } \theta = \arctan \frac{H}{R} \] Where \( \theta \) is the angle of repose, \( R \) being the radius of the conical pile.

Hausner ratio
It is related to interparticle friction and as such can be used to predict the powder flow properties. Powders with low interparticle friction such as coarse spheres have a ratio of approximately 1.2, whereas more cohesive, less flowable powders such as flakes have a Hausner ratio greater than 1.6. The equation for measuring the Hausner ratio is: \( \frac{D_f}{D_o} \), where \( D_f \) = Tapped density and \( D_o \) = Bulk density.

Table 5: Physical characteristic and Sodium contents (%) of different formulations of sitopaladi churna

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Formulation A</th>
<th>Formulation B</th>
<th>Formulation C</th>
<th>Formulation D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap density</td>
<td>0.6±0.03</td>
<td>0.421±0.01</td>
<td>0.05±0.48</td>
<td>0.521±0.002</td>
</tr>
<tr>
<td>Bulk density</td>
<td>0.35±0.04</td>
<td>0.407±0.01</td>
<td>0.316±0.03</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>Angle of repose</td>
<td>26±0.23</td>
<td>32.5±0.03</td>
<td>24.87±0.86</td>
<td>32.53±0.06</td>
</tr>
<tr>
<td>Hausner ratio</td>
<td>1.66±0.04</td>
<td>1.68±0.02</td>
<td>1.75±0.02</td>
<td>1.73±0.021</td>
</tr>
<tr>
<td>Sodium contents</td>
<td>4.3</td>
<td>4.6</td>
<td>4.8</td>
<td>3.71</td>
</tr>
</tbody>
</table>

*Samples are collected and labeled as Dabur, Zandu, Baidyanath, Inhouse. Named as formulation A, formulation B, formulation C, formulation D respectively

HPTLC finger printing profile
HPTLC study of methanolic extracts of the individual ingredients, in-house formulation and marketed formulations were carried out along with the different marker
compound corresponding to the active ingredients to ensure the presence of active ingredients in all the formulations. For HPTLC, 2gm of each sample (Formulation-A, B and C) and the in-house formulation(D) were extracted with 25ml of methanol on boiling water bath for 25 minutes consecutively three times using fresh potion of 25ml methanol, filtered and concentrated. The chromatograph was performed by spotting standards and extracted samples on pre coated silicagel aluminium plate 60F-254 (10cm×10cm with 250µm thickness) using Camag Linomat IV sample applicator and 100µl Hamilton syringe. The samples, in the form of bands of length 5mm, were spotted 15mm from the bottom, 10mm apart, at a constant application rate of 15nl/s using nitrogen aspirator. Plates were developed using mobile phase consisting of toluene-ethylacetate. Subsequent to the development, TLC plates (in Fig 1a-d.) were dried in a current of air with the help of an air-dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance/reflectance mode. The HPTLC fingerprint profiles of the formulations are presented in Fig 2a-d. [11,12]

Figure 1a: TLC fingerprint profile of α- terpinyl acetate & Sitopaladi formulation
Track1: Formulation D
Track2: Formulation A
Track3: Formulation C
Track4: α- terpinyl acetate
Track5: Elettaria cardamomum
Track6: Formulation B
Solvent System: Toluene : Ethyl acetate (19.5 : 0.5)

Figure 1b: TLC fingerprint profile of Piperine & Sitopaladi formulation
Track1: Formulation D
Track2: Formulation A
Track3: Formulation C
Track4: – Piperin
Track5: Piper longum
Track6: Formulation
Solvent System: Toluene : Diethyl ether : Dioxane(( 62.5 : 21.5 : 16))
Figure 1c: TLC fingerprint profile of Cinnamaldehyde & Sitopaladi formulation
Track 1: Formulation D
Track 2: Formulation A
Track 3: Formulation C
Track 4: Cinnamaldehyde
Track 5: Cinnamomum zeylanicum
Track 6: Formulation B
Solvent System: Toluene : Ethyl acetate (19.5 : 0.

Figure 1d: TLC fingerprint profile of various formulation of Sitopaladi churna
Track 1: Sitopaladi Churna (Lab. Preparation)
Track 2: Sitopaladi Churna (Lab. Preparation)
Track 3: Sitopaladi Churna (Zandu)
Track 4: Sitopaladi churna (Dabur)
Track 5: Sitopaladi churna (Baidyanath)
Track 6: Sitopal churna (Lab. Preparation)
Solvent System: Toluene : Diethyl ether : Formic acid (5 : 2.5 : 0.5)
Fig: 2c
HPTLC of Sitopaladi Churna (Zandu)

Fig: 2d
HPTLC of Sitopaladi Churna (Baidyanath)

Fig: 3a Vasmolochana
Silica crystals

Fig: 3b Piper longum

Fig: 3c Eletteria cardamomum

Fig: 3d Cinnamomum zeylanicum

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RESULTS AND DISCUSSION

In house formulation was prepared in accordance with the Ayurvedic Formulary of India. As part of standardization procedure, the finished product Sitopaladi Churna was tested for relevant physical and chemical parameters along with samples from three different manufacturers, D, B and Z for a comparative study. All the samples were whitish brown in color. The powders were smooth, having fragrant odor, possessing Sweetish sweet taste. The organoleptic properties of the marketed formulations and the in-house formulations were reported in table 1. Quality tests for different Sitopaladi Churna and its individual ingredients were performed for moisture content, ash content, water soluble extractive, methanol soluble extractive, acid insoluble ash and water insoluble ash, and were found to be within standard ranges. The extractive values and ash values of individual ingredients of churna, in-house formulation and different marketed formulations are given in table 2. The results are expressed as mean (n=6) ± Standard deviation (SD). Variations were observed in most of the physicochemical parameters studied. The total ash value of formulation A was found to be higher than that for B, C and D. Acid insoluble ash value for in-house formulation (D) was found to be 22.3±0.41 and in case of marketed formulation A, B and C this was found to be 16.9±0.07, 20.6±0.31 and 21.0±0.04 respectively. On the contrary, water soluble ash percentage of A and B, D were comparable except C which was comparatively high. The extractive values of formulations in water were found to be much higher than alcohol extractive values. Loss on drying at (105°C) and pH of 1% w/v and 10% w/v aqueous solution are also presented in Table 3. pH of 1% and 10% w/v solution revealed that the formulations are basic in nature. In fluorescence analysis the powder samples were exposed to ultraviolet light at wavelength of 254nm and 366nm and to daylight after being treated with different reagents as reported in table 4. Fluorescence analysis results shows whether any fluorescent ingredients are present or not, here we have found there was no such material found in any of formulation and individual ingredients either. The physical characteristics of the in-house formulation (D) and two market formulations (average value along with standard deviation) are shown in Table 5. The results of the market formulations and in house formulation were found to be comparable. The flowability of the formulation was found to be poor in both market formulation and in house formulation, which was further confirmed by high values of Hausner ratio. For the estimation of sodium by flame photometer the emission intensity of different concentrations are presented in table 5. The sodium content was found to be less in in-house formulation (3.71%) and highest in Baidyanath formulation (4.8%). T.L.C & H.P.T.L.C study of individual ingredient and formulations was calculated the presence ingredient. The marker compound cinnamaldehyde, Piperine, α-terpinyl acetate A wer estimated by HPTLC in

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sitopaladi churna sample .Rf 0.44 correspondin cinnamaldehyde,RF 0.78 corresponding to α-terpinyl acetate,RF 0.5 corresponding to piperine, these are visible in both the ingredient and formulation. HPTLC fingerprint profile of the Sitopaladi Churna formulations are depicted in figure 1a-d indicates the presence of all the ingredients in proportional quantity in the formulations. This also confirms the brand-to-brand consistency of the finished product. Ayurvedic medicine Sitopaladi Churna has been standardized by intervention of modern scientific quality control measures described in classical texts. Hence, the physicochemical parameters, quantitative analysis, & HPTLC fingerprint profiles together may be used for quality evaluation and the standardization of compound formulations. Powder microscopy of sitopaladi churna & individual ingredient was done it shows different structure.silica crystals(vamsalochan),epidermis of the testa(elettaria cardamom),oil of testa (elettaria cardamom),sclerids(cinnamomum zeylanicium),perisperm cells containing compacted masses of starch grains(piper longum) these structure present in sitopaladi churna formulations which shown in fig-3a-e.

CONCLUSION

Ayurvedic medicin sitopaladi churna has been standardized by intervention of scientific quality control measures in the traditional preparation describe in classical texts.pharmacognostic characters established for the raw material could be employed as Q.C, standards for evaluating its identity and can be used for routine analysis.of Purity and potency of the material and formulations following procedure given could be performed in QC/QA labotary of pharmaceutical house.

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