Evaluation of Quality Parameters of Three Different Marketed Brands of Yogaraj Guggulu Vati: A Polyherbal Formulation

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Abstract

Yogaraj Guggulu Vati is one of the most commonly used polyherbal formulation in Ayurvedic clinical practice since ages for the ailments of various bone disorders.

Objective: The present work was carried out in order to evaluate comparatively three different and popular formulations of Yogaraj Guggulu Vati to confirm their quality and also to highlight the variations that may be present in them.

Methods: Comparative assessment of three different and popular marketed formulations of Yogaraj Guggulu Vati was carried out for their organoleptic, pharmaceutical, physicochemical and phytochemical properties as per the methods prescribed in Pharmacopoeias.

Results: The observations reveal that all the parameters of three brands of Yogaraj Guggulu Vati had considerable differences in their values. The value of water soluble and alcohol soluble extractives of Brand A was very much higher and lesser than the standard values respectively. The pH of all the tablets was also higher than that of the standard values. The Hardness and Disintegration time of the tablets of Brand B was also very high than the other two brands. The tablets of Brand A also failed the weight variation test.

Conclusion: It can be concluded from the overall results that phytochemical and analytical evaluation and standardization of all the formulations should be performed strictly on all formulations so as to optimize the final product according to the standards, which would otherwise affect the therapeutic activity of the product.

Keywords: Quality Evaluation; Yogaraj Guggulu Vati; Pharmaceutical Evaluation; Physico-Chemical Evaluation; Phytochemical Evaluation; Heavy Metal Analysis

Abbreviations: API: Ayurvedic Pharmacopoeia of India; IP: Indian Pharmacopoeia; Pb: Lead; Cd: Cadmium

Introduction

Guggulu is a herb popular in Ayurveda which is used to treat various ailments. It is an oleogum resin that spontaneously exudes due to the injury of the bark of Commiphora wightii. Guggul, the Commiphora mukul, is a small thorny tree native to the Middle East and the Indian plains. The name Guggul means “one that protects from disease”. Guggul is one of the “broad spectrum” health products with a wide range of benefits. There are five different varieties of Guggulu described in Ayurvedic texts. However, two of the varieties, namely, Mahisaksa and Kanaka Guggulu are generally preferred for medicinal preparations. Mahisaksa Guggulu is dark greenish brown and Kanaka Guggulu is yellowish brown [1-3]. Yogaraj guggulu is a polyherbal formulation consisting of 29 ingredients with Guggulu as the major one. It acts by vitiating the Tridoshas. It is used traditionally for detoxifying, treating obesity, joint pain, arthritic conditions, muscle aches, rheumatism and gout. It increases digestion power, complexion, strength and immunity [2,4].

Quality control of the herbal formulation and drug is important to justify its acceptability in the modern drug system. The change from batch to batch begins with the collection of the raw materials themselves in the absence of any reference standard for identification. WHO has emphasized the need to ensure quality control of medicinal plant products using modern techniques and applying appropriate standards and parameters. The present work was carried out to standardize and evaluate the pharmaceutical, physicochemical and pharmacognostical properties of three different marketed brands of Yogaraj Guggulu Vati.
Materials and Methods [5-13]

Procurement of Samples

The following marketed Yogaraj Guggulu Vati preparations were used in the present study: Brand A (Batch No. SB 0528), Brand B (Batch No. E-1601), Brand C (Batch No. H-1-254). All brands of the Yogaraj Guggulu Vati were procured from the local market from the registered Ayurvedic Pharmacy.

Organoleptic Evaluation

All the organoleptic properties viz. color and odor of the drug were performed as per standard procedure and noted down. Table 1: Weight Variation Limits as per Various Pharmacopoeias.

| IP/BP       | Limit          | USP          |
|-------------|----------------|--------------|
| 80 mg or less | ± 10%          | 130 mg or less |
| 80 - 250 mg  | ± 7.5%         | 130 - 324 mg |
| >250 mg      | ± 5%           | >324 mg      |

Determination of Moisture Content / Loss on Drying (LOD): The moisture content of the dry drug was determined by weighing it in a tared dish. The sample was then placed in the muffle furnace at 105°C overnight (or previously dried sample can also be used). The sample was then placed in the muffle furnace at 105°C for 3 hours. The sample was then cooled in a desiccator for 30 min and reweighed with the ash in it (W3). The observations were noted.

Formula for calculation:
% LOD = (W2 - W1)/(W3 - W1) × 100 %
Where,
W1 = weight of container + wet sample (g)
W2 = weight of container + dried sample (g)
W3 = weight of moisture
W4 = weight of dried sample

%LOI=(W5 - W4)/(W5 - W2) × 100 %
Where,
W2 = weight of crucible (g)
W4 = weight of sample (g)
W5 = weight of ash (g)

Determination of Total Ash: An accurately weighed 3 g of the sample was taken in a previously ignited and tared silica crucible and was heated in the oven at 105°C overnight (or previously dried sample can also be used). The crucible was cooled and reweighed. The crucible was then placed into the furnace tray and was ignited in the Muffle Furnace at 500°C for about 4 hrs. The sample was then cooled in a desiccator for 30 min. and reweighed with the ash in it (W4). The observations were noted.

Determination of Disintegration Time: For the disintegration test one tablet was placed in each tube, the guide disc was placed above each tablet and the basket rack was positioned in 1 L beaker containing water at 37°C±2°C. For compliance with USP standards the tablets must disintegrate and all particles must pass through the 10 mesh screen in the specified time. If any residue remains in the case of Ayurvedic tablets the disintegration time required for five tablets should not be more than 15 minutes, unless otherwise stated in the monograph.

Physico-Chemical Evaluation

Physicochemical parameters like moisture content (Loss on Drying), pH, total ash, acid Insoluble ash, water-soluble extractive, alcohol soluble extractive values of all three samples were determined as per standard protocols. All the procedures are described as follows:

Determination of Moisture Content / Loss on Drying (LOD): An accurately weighed 5g of polyherbal formulation powder was taken in a tared evaporating dish. The crude drug was then heated at 105°C in an oven for 3 hours. The drying and weighing was continued at half an hour interval until difference between two successive weighing corresponded to, not more than 0.25 per cent. Percentage moisture content of the sample was calculated with reference to the air dried powdered drug material.

Formula for calculation:
% LOD = (W2 - W1)/(W3 - W1) × 100 %
Where,
W1 = weight of container (g)
W2 = weight of crucible (g)
W3 = weight of sample (g)
W4 = weight of ash (g)

Determination of Total Ash: An accurately weighed 3 g of the sample was taken in a previously ignited and tared silica dish/crucible. The material was evenly spread and ignited in a Muffle Furnace by gradually increasing the temperature to not more than 450°C - 600°C till the carbon free ash was not obtained. The Total Ash value was calculated with reference to the air-dried powdered drug material.

Formula for calculation:
% Total Ash = Weight of Ash/Weight of sample taken × 100 %

Determination of Acid Insoluble Ash: Ash above obtained, was boiled for 5 min with 25 ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble

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ash was calculated with reference to the air-dried powdered drug material.

Formula for calculation:
% Acid-Insoluble Ash = Weight of acid in soluble residue/ Weight of sample taken × 100%

Determination of Water Soluble Ash: 1g of ash obtained in total ash experiment was boiled for 5 min with 25 ml water and insoluble matter collected on an ashless filter paper which was then washed with hot water and ignited for 15 min at a temperature not exceeding 450°C in a muffle furnace. Difference in weight of ash and weight of insoluble matter was determined as difference represents the value. The percentage of water insoluble ash was calculated with reference to the air-dried powdered drug material.

Formula for calculation:
% Water Soluble Ash = Weight of water soluble residue/ Weight of sample taken × 100%

Determination of Extractive Values

(a) Determination of Alcohol Soluble Extractives: 5 gm of churna was accurately weighed and placed inside a glass stoppered conical flask. It was then macerated with 100 ml of ethanol. The flask was shaken frequently during the first 6 hours and was kept aside without disturbing for 18 hours. It was then filtered and about 25 ml of filtrate was transferred into a tared flat-bottomed shallow dish and was evaporated to dryness on a water bath. It was then dried to 105°C for 6 hours, cooled and finally weighed. The percentage of Alcohol Soluble extractives was calculated with reference to the air-dried powdered drug material.

Formula for calculation:
% Alcohol Soluble Extractive = (Weight of residue × 100 × 100) /
(25 × Weight of sample taken) %

(b) Determination of Water Soluble Extractives: Proceed as directed for determination of Alcohol-Soluble Extractive, using chloroform water (2.5 ml chloroform in purified water to produce 1000 ml) instead of ethanol.

(c) Determination of pH Value: The powder sample of Yogaraj Guggulu Vati was weighed to about 5g and immersed in 100 ml of water in a beaker. The beaker was closed with aluminum foil and left behind for 24 hours in room temperature. Later the supernatant solution was decanted into another beaker and the pH of the formulation was determined using a calibrated digital pH meter.

(d) Phytochemical Evaluation

The aqueous and alcoholic extracts of the respective formulations were prepared and were subjected to preliminary phytochemical screening. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. Methods for preliminary qualitative phytochemical tests of the plant extracts are given below in the Table 2.

Table 2: Preliminary Phytochemical Tests for Plant Extracts.

| S.No. | Phyto-Constituents | Name of Tests | Procedure | Observation |
|-------|--------------------|---------------|-----------|-------------|
| 1     | Alkaloids          | Mayer’s test  | 2 ml extract + few drops of HCl + Mayer’s reagent | Cream Precipitation |
|       |                    | Hager’s test  | 2 ml extract + few drops of HCl + Hager’s reagent | Yellow Precipitation |
|       |                    | Wagner’s test | 2 ml extract + few drops of HCl + Wagner’s reagent | Reddish brown color |
| 2     | Carbohydrates      | Molish test   | 2 ml extract + 2 Drops of Molish reagent + few drops of Conc. H_2SO_4 | Violet or Reddish color |
| 3     | Reducing sugars    | Fehling’s test| 1 ml extract + 1 ml Fehling Solution (A and B) | First a Yellow and then Brick Red Precipitation |
| 4     | Flavonoids         | Alkaline reagent test | 2 ml extract + few drops of 40% NaOH solution | Intense yellow color forms which becomes colorless on addition of dil. acid |
|       |                    | Lead acetate test | 2 ml extract + few drops of Lead Acetate solution | Yellow precipitation |
| 5     | Saponins           | Foam test     | 2 ml extract + 4 ml distilled H_2O Mix well and shake vigorously | Foam formation |
| 6     | Tannins            | Braymer’s test| 2 ml extract + 2 ml H_2O + 2-3 drops of 5% FeCl_3 | Black green or bluish color |
| 7     | Steroids           | Salkowski’s test | 2 ml extract + 2 ml Chloroform + 2 ml Conc. H_2SO_4 | Chloroform layer appears red and acid layer shows greenish-yellow fluorescence |
| 8     | Proteins           | Millon’s test | 3 ml extract + 5 ml Millon’s reagent | White precipitate which turns brick red on warming |
| 9     | Glycosides         | Keller Killiani’s test | 2 ml extract + Glacial acetic Acid + 1 drop of 5% FeCl_3 + Conc. H_2SO_4 | Reddish brown color appears at the junction of 2 layers and upper layer appears bluish green |
| 10    | Phenols            | -             | 2-3 ml of extract + few drops of 5% FeCl_3 solution | Deep blue-black color |
|       |                    |               | 2-3 ml of extract + few drops of Lead Acetate solution | White precipitate |
| 11    | Amino acids        | Ninhydrin test | 3 ml of extract + 3 drops of 5% Ninhydrin solution Keep in boiling water bath for 10 min. | Purple or bluish color appears |
| 12    | Terpenoids         | Copper Acetate test | 2 ml extract dissolved in water + 3-4 drops of Copper Acetate solution | Emerald green color |

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Determination of Heavy Metals (Lead and Cadmium)

Method (Direct Calibration Method): Three reference solutions of the element being examined having different concentrations were prepared covering the range recommended by the instrument manufacturer. Separately the corresponding reagents were added for the test solution and the blank solution was prepared with the corresponding reagents. The absorbance of the blank solution and each reference solution were measured separately, and the readings were recorded. A calibration curve was prepared with the average value of 3 readings of each concentration on the ordinate and the corresponding concentration on the abscissa. A test solution of the substance being examined was prepared as specified in the monograph. The concentration was adjusted such that it falls within the concentration range of the reference solution. The absorbance was measured 3 times, and the readings were recorded and the average value was calculated. The mean value was interpolated on the calibration curve to determine the concentration of the element.

Preparation of Lead Standard Solution: Lead standard solutions were prepared from Stock solution (1000 ppm Sisco Research Laboratories Pvt. Ltd. stock solution). Standard solutions of concentrations, 2, 4, 6, 8 and 10 ppm were prepared. The absorption of standard solution measured at 217 nm using hollow cathode lamp as a light source & air acetylene blue flame on Atomic absorption Spectrophotometer.

Preparation of Cadmium Standard Solution: Cadmium standard solutions were prepared from Stock solution (1000 ppm Sisco Research Laboratories Pvt. Ltd. stock solution). Standard solutions of concentrations 0.2, 0.4, 0.6, 0.8 and 1.0 ppm was prepared. The absorption of standard solution measured at 228.8 nm using hollow cathode lamp as a light source & air acetylene blue flame on Atomic absorption Spectrophotometer.

Preparation of Test Solution: Weigh accurately about 0.5 g of the coarse powder of the substance being examined, transfer into a casparian flask, add 5-10 ml of the mixture of nitric acid (HNO₃) and perchloric acid (HClO₄) (4:1), add a small hopper on the flask-top, macerate overnight, heat to slake on the electric hot plate, keep somewhat-boiling, if brownish-black in color, add again a quantity of the above mixture, continuously heat till the solution becomes clear and transparent, then raise temperature, heat continuously to thick smoke, till white smoke disperse, the slaked solution becomes colorless and transparent or a little yellow, cool, transfer it into a 50 ml volumetric flask, wash the container with 2% nitric acid solution (HNO₃), add the washing solution into the same volumetric flask and dilute with the same solvent to the volume, shake well. Prepare synchronously the reagent blank solution according to the above procedure.

Determination: Measure accurately 1 ml of the test solution and its corresponding reagent blank solution respectively, add 1 ml of solution containing 1% NH₄H₂PO₄ and 0.2% Mg(NO₃)₂, shake well, pipette accurately 10-20 μl to determine the absorbance.

Sample Analysis: The analysis of the digested samples were carried out using an Atomic Absorption Spectrophotometer (EC Electronics Corporation of India limited AAS Element AS AAS4141) for Lead and Cadmium. The instrumental conditions for Lead analysis are depicted in Table 3.

Table 3: Instrumental Conditions for Analysis of Lead and Cadmium.

| Parameters       | Pb     | Cd     |
|------------------|--------|--------|
| Wavelength (nm)  | 217    | 228.8  |
| Slit width (nm)  | 1.0    | 0.5    |
| Light Source     | Hollow Cathode Lamp | Hollow Cathode Lamp |
| Flame type       | Air/C₂H₂ | Air/C₂H₂ |
| Current          | 10     | 3.5    |
| AAS Technique    | Flame  | Flame  |

Results

Organoleptic Evaluation

The observations for the organoleptic evaluation of three brands of Yogaraj Guggulu Vati are reported in Table 4.

Table 4: Results for Organoleptic Evaluation of different brands of Yogaraj Guggulu Vati.

| S.No. | PROPERTIES | BRAND A         | BRAND B         | BRAND C         | STANDARD (API-II Vol.2) |
|-------|------------|-----------------|-----------------|-----------------|------------------------|
| 1     | Description| Solid Tablets   | Solid Tablets   | Solid Tablets   | -                      |
| 2     | Color      | Greyish Brown   | Blackish Brown  | Dark Brown      | Dark Brown             |
| 3     | Odor       | Characteristic  | Characteristic  | Characteristic  | Spicy Pleasant         |
Pharmaceutical Evaluation

The observations for the pharmaceutical evaluation of three brands of Yogaraj Guggulu Vati are reported in Table 5.

**Table 5: Results for Pharmaceutical Evaluation of different brands of Yogaraj Guggulu Vati.**

| S.No. | PROPERTIES     | BRAND A | BRAND B  | BRAND C  | STANDARD (API-II Vol.2) |
|-------|----------------|---------|----------|----------|-------------------------|
| 1.    | Weight Variation | Failed  | Passed   | Passed   | -                      |
| 2.    | Thickness       | 4.92±0.23 | 4.75±0.057 | 6.60±0.082 | -                      |
| 3.    | Diameter        | 9.76±0.02 | 8.12±0.074 | 10.88±0.02 | -                      |
| 4.    | Hardness        | 3.83±0.289 | 13.83±0.289 | 1.67±0.289 | -                      |
| 5.    | Disintegration Time | 57 min. | 85 min   | 52 min  | NMT 60 min             |

Physico-Chemical Evaluation

The observations for the physico-chemical evaluation of three brands of Yogaraj Guggulu Vati are reported in Table 6

**Table 6: Results for Physico-chemical Evaluation of different brands of Yogaraj Guggulu Vati.**

| S.No. | PROPERTIES                        | BRAND A | BRAND B | BRAND C | STANDARD (API-II Vol.2) |
|-------|-----------------------------------|---------|---------|---------|-------------------------|
| 1.    | pH                                | 7.1     | 5.2     | 5.8     | 4.7-5.0                 |
| 2.    | Loss on Drying/ Moisture Content  | 6.39%   | 7.53%   | 5.94%   | NMT 10%                 |
| 3.    | Water Soluble Extractive          | 30.4%   | 20.8%   | 24.8%   | NLT 19%                 |
| 4.    | Alcohol Soluble Extractive        | 4.8%    | 5.6%    | 11.2%   | NLT 16%                 |
| 5.    | Loss on Ignition                  | 78.33%  | 91.24%  | 84.96%  | -                      |
| 6.    | Total Ash Value                   | 5.32%   | 5.58%   | 5.49%   | NMT 6.0%                |
| 7.    | Acid Insoluble Ash                | 0.47%   | 0.56%   | 0.46%   | NMT 1.0%                |
| 8.    | Water Soluble Ash                 | 2.1%    | 2.54%   | 1.52%   | -                      |

Phytochemical Evaluation

The observations for the phytochemical evaluation of three brands of Yogaraj Guggulu Vati are reported in Table 7

**Table 7: Phytochemical Screening of Yogaraj Guggulu Vati.**

| S. No. | PHYTO-CONSTITUENT | NAME OF TESTS | BRAND A | BRAND B | BRAND C |
|--------|-------------------|---------------|---------|---------|---------|
| 1.     | Alkaloids         | Hager’s test  | -       | +       | -       |
|        |                   | Wagner’s test | -       | +       | -       | +       |
|        |                   | Mayer’s test  | -       | +       | -       | +       |
| 2.     | Glycosides        | Keller Killani’s test | -       | -       | +       | +       |
| 3.     | Carbohydrates     | Molish’s test | +       | +       | +       | +       | +       |
| 4.     | Proteins          | Biuret’s test | -       | -       | -       | -       | -       |
| 5.     | Amino Acids       | Ninhydrin’s test | -       | -       | -       | -       | -       |
| 6.     | Steroids          | Salkowski’s test | ++      | ++      | +       | +       | +++     | +++     |
| 7.     | Flavonoids        | Alkaline Reagent test | -       | -       | -       | -       | -       |
|        |                   | Lead acetate test | -       | -       | -       | -       | -       |
| 8.     | Terpenoids        | Copper Acetate test | +       | +       | +       | +       | +       |
| 9.     | Tannins           | Ferric Chloride test | +       | +       | +       | +       | +       |
| 10.    | Saponins          | Foam test     | -       | -       | -       | -       | -       |
| 11.    | Phenols           | Ferric Chloride test | -       | -       | +       | -       |
|        |                   | Lead Acetate test | -       | -       | +       | -       | -       |
Determination of Heavy Metals (Lead and Cadmium)

The observations for the heavy metal determination of three brands of Yogaraj Guggulu Vati are reported in Table 8 and Graph 1.

Graph 1: Graphs for various Pharmaceutical and Physico-chemical parameters of different brands of Yogaraj Guggulu Vati.
**Discussion**

Yogaraj Guggulu vati of Brand A was of solid state of Greyish brown color. This preparation had pH value of 7.1, and Loss on drying value of 6.39% w/w. Preparation had Alcohol soluble extractives and Water soluble extractives values of 4.8% w/w and 30.4% w/w respectively. The tablets failed the Weight variation test as per IP standards. The mean value and standard deviation of Thickness and Diameter of the tablets were 4.92 ± 0.23 and 9.76 ± 0.02 respectively. The tablet had Hardness value of 3.83 ± 0.289. The Disintegration time of the tablets was 57 min. respectively. It had Total Ash value of 5.32% w/w, and Acid insoluble ash and Water soluble ash value of 0.47% w/w and 2.1% w/w respectively. Loss on ignition was found 78.33% w/w. The concentrations for heavy metals Lead and Cadmium were found to be 1.762 and 0.189 respectively which were within the prescribed limits. Physiochemical screening revealed the presence of Carbohydrates, Steroids, Tannins and Terpenoids in both the extracts and of Alkaloids in alcoholic extract only.

Yogaraj Guggulu vati of Brand B was of solid state of Blackish brown color. This preparation had pH value of 5.2, and Loss on drying value of 7.53% w/w. Preparation had Alcohol soluble extractives and Water soluble extractives values of 5.6% w/w and 20.8% w/w respectively. The tablets passed the Weight variation test as per IP standards. The mean value and standard deviation of Thickness and Diameter of the tablets were 4.75 ± 0.057 and 8.12 ± 0.074 respectively. The tablet had Hardness value of 13.83 ± 0.289. The Disintegration time of the tablets was 85 min. respectively. It had Total Ash value of 5.58% w/w, and Acid insoluble ash and Water soluble ash value of 0.56% w/w and 2.54% w/w respectively. Loss on ignition was found 91.24% w/w. The concentrations for heavy metals Lead and Cadmium were found to be 2.923 and 0.257 respectively which were within the prescribed limits. Physiochemical screening revealed the presence of Carbohydrates, Steroids, Tannins and Terpenoids in both the extracts; Phenols in aqueous extract only and of Alkaloids in alcoholic extract only.

Yogaraj Guggulu vati of Brand C was of solid state of Dark brown color. This preparation had pH value of 5.8, and Loss on drying value of 5.94% w/w. Preparation had Alcohol soluble extractives and Water soluble extractives values of 11.2% w/w and 24.8% w/w respectively. The tablets passed the Weight variation test as per IP standards. The mean value and standard deviation of Thickness and Diameter of the tablets were 6.60 ± 0.082 and 10.88 ± 0.02 respectively. The tablet had Hardness value of 1.67 ± 0.289. The Disintegration time of the tablets was 52 min. respectively. It had Total Ash value of 5.49% w/w, and Acid insoluble ash and Water soluble ash value of 0.46% w/w and 1.52% w/w respectively. Loss on ignition was found 84.96% w/w. The concentrations for heavy metals Lead and Cadmium were found to be 2.981 and 0.234 respectively which were within the prescribed limits. Phytochemical screening revealed the presence of Carbohydrates, Steroids, Tannins and Terpenoids in both the extracts and of Alkaloids in alcoholic extract only.

**Conclusion**

Thus, all the parameters of three brands of Yogaraj Guggulu Vati had considerable difference in their values. The value of water soluble and alcohol soluble extractives of Brand A i.e. 30.4% and 4.8% was very much higher and lesser than the standard values of 19% and 16% respectively. The pH of all the tablets was also higher than that of the standard values. The Hardness and Disintegration time of the tablets of Brand B was also very higher than the other two with the value of 13.83 ± 0.289 and 85 min. respectively. The tablets of Brand A also failed the weight variation test.

The results of the present study clearly indicate that there is no uniformity in preparation of formulations. Organoleptic evaluation shows less similarity between formulations; Physicochemical analysis data depicts there are considerable differences in the quality parameters of all formulations and some parameters even don’t comply with the standard values given in different Pharmacopoeias. Hence, all these factors lead to variation in efficacy of drug formulations.

Therefore, we conclude that there is an urgent need to make more stringent quality control parameters and standardize the procedures for drug formulations from different reference books in order to reduce variation among all the Ayurvedic preparations.

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