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Formulation and evaluation of antistress polyherbal capsule

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ABSTRACT

The most important challenges faced by herbal formulations arise because of their lack of complete evaluation. So evaluation is necessary to ensure the quality and purity of the herbal product. For evaluation of capsule containing poly herbal crude drugs various parameters were tested. The aim of present study was to formulate and evaluate the polyherbal capsule and evaluate it for parameter of raw material include physicochemical studies like ash values and extractive values. Preformulation parameters and parameters for finished product (hard gelatin capsule) include uniformity of weight, disintegration time and other quality control test were performed. HPTLC and DSC was done for marker compounds, individual drugs and finished product. Data of HPTLC studies suggests that extract was derived from genuine plant parts. DSC thermo gram suggested that there was no interaction between the crude drugs and excipients. Future prospects include the clinical trials of the finished product as the clinical efficacy is already proven in different animal studies.

Keywords: Antistress Polyherbal capsule, Quality control, HPTLC, DSC.

INTRODUCTION

Herbal medicine is the oldest form of health care known to mankind. It is an integral part of the development of modern civilization. In herbal medicine plant based formulation are used to alleviate diseases. But the most important challenges faced by these formulations arise because of their lack of complete evaluation. So evaluation is necessary to ensure the quality and purity of the herbal product. It is very important to establish a system of evaluation for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous [1].

In poly-herbal preparations it will be very difficult if we want to estimate each and every ingredient in term of their chemical constituent. But if few major constituents having particular therapeutic action indicated in the labeled can be pinpointed then these constituents should be estimated quantitatively along with the other parameters through which presence of all ingredients can be confirmed.

Stress refers to inappropriate physiological response to any demand. It covers a wide range of phenomena, from mild irritation to drastic dysfunction that may cause severe health breakdown.

In other words, stress is considered to be any condition which results in perturbation of the body's homeostasis. If the level of stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, viz. hypertension, peptic ulcer, diabetes, immunosuppression, reproductive and behavioral disorders [2].

Herbal formulations enhances physical endurance, mental functions and non-specific resistance of the body and have been termed s Adaptogens. The potential utility of safer and cheaper herbal medicines as antistress agents have been reported as they can withstand stress without altering the physiological functions of the body. Various herbs like *Withania somnifera, Emblica officinalis, Asparagus racemosus, Ocimum sanctum, Tribulus terrestris* and *Piper longum* are claimed to have immunomodulatory, adaptogenic, anabolic effects and the ability to improve vital energy [3]. With this background the present study has been undertaken to prepare polyherbal formulation having antistress activity.

MATERIALS AND METHODS

Plant material

Fresh samples of four plants namely *Withania somnifera, Tinospora cordifolia, Emblica officinalis* and *Eugenia caryophyllus* were purchased from local markets. The plant was dried under shade to a constant weight and coarsely powdered in a electronic mixer, sieved through mesh no. 40 and stored in air tight, well closed container till further use.

Physicochemical studies

Physicochemical studies like total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble extractive, alcohol soluble extractive and loss on drying were determined to check the purity of the plant material [4, 5].

Preformulation parameters

Bulk density and tap density and Carr's index [6, 7]

A weighed quantity (15g) of powdered material was taken in a 50ml measuring cylinder. And recorded the initial volume (v_o). tapped the contents and recorded the powdered volumes after 50 taps(v_{50}).

Fluff density = w/v_o g/cc
Tapped density = w/v_{o50} g/cc
Carr's index = Tapped density- Fluff density/ Tapped density * 100

Value for Carr's index below 15 indicate excellent flowing material and value over 20-30 suggested poor flowing material.

Angle of repose [8]

A funnel was fixed at a particular height (1.5, 2.5, 3.5 cm) on a burette stand. A white paper was placed below the funnel on the table. The powdered drug passed slowly through the funnel until it forms a pile. The radius of the pile was noted down.

Angle of repose of the powder material was calculated by using the formula:

$\tan\theta = h/r$

$\theta = \tan (h/r)$

where, h = height of the pile, r = radius.

Values for angle of repose $< 30^o$ usually indicate a free flowing material and angle $> 40^o$ suggest a poor flowing material.

Hausner's ratio [8]
The basic procedure is to measure the unsettled apparent volume, V_0 and the final tap volume, V_f, of the powder tapping the material until no further volume changes occur. The Hausner's ratio was calculated as follows:

Hausner's ratio = V_0 / V_f

Hausner's ratio between 1.00 to 1.11 shows excellent flow and value more than 1.60 shows very, very poor flow.

Quality control parameters [7, 9]
Uniformity of weight
Test for uniformity of weight was performed as per Indian pharmacopoeia, 2010.

Disintegration test
Disintegration test was performed using the digital microprocessor based disintegration test apparatus (Electro lab, Mumbai).

One capsule was introduced into each tube and added a disc to each tube. The assembly was suspended in the water in a 1000 ml beaker. The volume of water was such that the wire mesh at its highest point is at least 25 mm below the surface of the water, and at its lower point was at least 25 mm above the bottom of the beaker. The apparatus was operated and maintained the temperature at 37±2°C. (Indian Pharmacopoeia, 2010).

Composition of capsule
Each 500mg capsule contains:

Withania somnifera	125mg
Tinospora cordifolia	125mg
Emblica officinalis	125mg
Eugenia caryophyllus	125mg
Excipients	*q.s.*

HPTLC finger-printing of raw material and finished product [10, 11]
HPTLC was done with pre-coated aluminum plates with Silica Gel $60F_{254}$ (Merck, India) of 10 x 10 cm and 0.2 mm thickness. CAMAG LINOMAT IV was used for sample application.

Detection was done at UV 366nm and 254 nm using CAMAG TLC scanner. R_f of compounds were matched and respective area was measured.

Preparation of formulation by Wet granulation method [12]
Withania somnifera, Tinospora cordifolia, Emblica officinalis and *Eugenia caryophyllus* were finely powdered (# 40), and taken for preparation of capsules by wet granulation technique using starch (20%) solution as binder. The wet mass was passed through # 30 to obtain granules. The granules were dried at 45^{o}C in tray dryer. The granules were lubricated with 1% magnesium stearate. Diluents and preservatives were added and filled in capsules colored yellow – red size '00' in capsule filling machine. The capsules were evaluated for weight variation content uniformity and disintegration time.

RESULTS AND DISCUSSION

Physicochemical parameters
Various physicochemical parameters were calculated for the herbal drugs used in the polyherbal formulation. Table 1 depicts the report of various physicochemical parameters.

Table 1. Physicochemical parameters

S. No.	Physicochemical parameters	*Withania somnifera*	*Tinospora cordifolia*	*Emblica officinalis*	*Eugenia caryophyllus*
1	Total ash value	4.89	7.15	3.92	5.34
2	Acid insoluble ash value	0.17	0.28	0.75	0.56
3	Water soluble ash value	3.67	3.9	2.41	2.79
4	Water soluble extractives	20.23%	25.2%	46.16%	28.08%
5	Alcohol soluble extractives	18.82%	15.71%	28%	11.92%
6	Loss on drying	5.23	2.56	4.35	3.61

Preformulation studies

Preformulation parameters like bulk density, tap density, Carr's index, Hausner's ratio and angle of repose were obtained for the laboratory granules. The granules showed excellent flow property.

Table 2. Preformulation parameters

S. No.	Parameters	Results
1	Bulk density	0.6
2	Tap density	0.8
3	Carr's index	19.6
4	Hausner's ratio	1.24
5	Angle of repose	14.03

Quality control tests

The polyherbal capsule contains four crude drugs namely *Withania somnifera*, *Tinospora cordifolia* , *Emblica officinalis* and *Eugenia caryophyllus* 125mg each. Six trials were taken. The final batch was tested for weight variation, uniformity of weight and disintegration time. During the stability studies the capsules were found to be stable throughout the period and there was no change in the shelf life of herbal capsules. The results are as follows in table 3 below.

Table 3. Quality control tests on anti-stress herbal formulation

S. No.	Parameters	Result
1	Weight variation	Within limits
2	Uniformity of weight	Within limits
3	Disintegration time	8 minutes 28 seconds
4	Loss on drying	3.94%

Differential scanning calorimetery [13, 14]

- The thermo grams of the samples were obtained by Differential Scanning Calorimeter (DSC Q-200, TA Instruments, USA) using scanning rate of 10^{o}C/min from 0^{o} to 400^{o}C. About 4-5mg of samples were taken and sealed in Aluminium pans and analysed under flow of nitrogen gas at the rate of 50ml/min. Figures 1-4 shows the thermo grams of individual crude drugs and antistress herbal formulation. The following information was obtained from the thermo grams of individual crude drugs and antistress herbal formulation.
- *E. officinalis* has Gallic acid as marker compound with a melting point of 250°C and our thermo gram showed the presence of Gallic acid at peak of 218°C without any interaction. The thermo gram is shown in fig. 3.
- *W. somnifera* has Withanolide A as marker is showing peak 252^{o}C and the standard melting point peak is 282^{o}C showing no signs of interaction with excipients. Fig. 4 presents the thermo gram of *W. somnifera.*
- *T. cordifolia* contains active constituent Berberine is showing peak 192.57^{o}C and the standard melting point peak is 197^{o}C- showing no signs of interaction with excipients. See fig. 1.
- *E. caryophyllus* which contains eugenol as major constituent was not detected in thermo gram because it is a volatile constituent and hence, thermo gram was not available.
- DSC thermo gram of antistress herbal formulation did not showed any interaction with excipients of the formulation. Fig 5 presents the DSC thermo gram of anti-stress formulation.

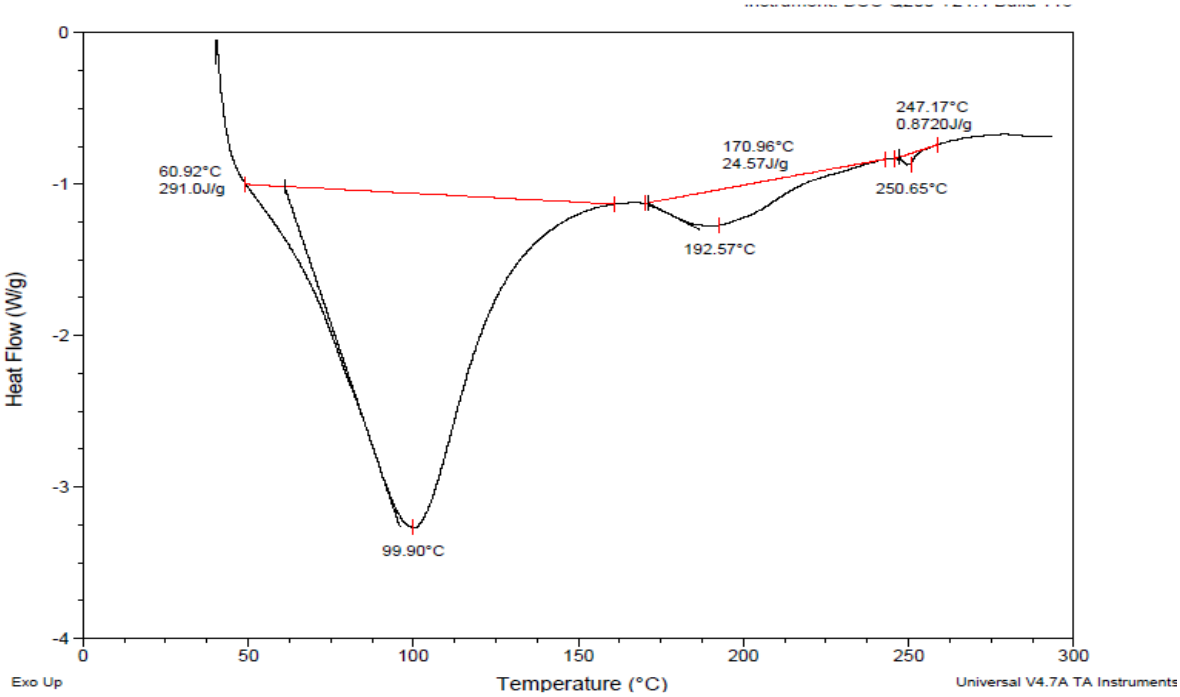

Fig. 1. Thermo gram of *T. cordifolia*

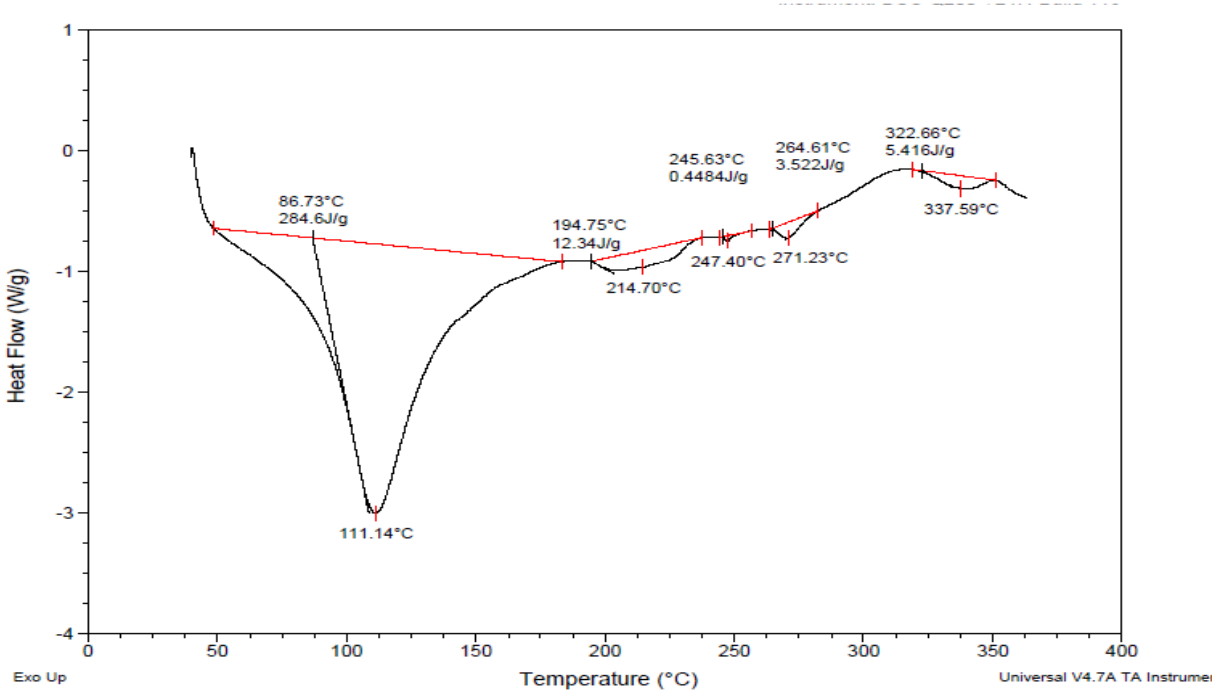

Fig. 2. Thermo gram of Antistress herbal formulation

Quantification by HPTLC

Quantification of eugenol, Withanolide A, Gallic acid and Berberine was done by HPTLC. And the total volatile content in clove (eugenol), Withanolide A, gallic acid and Berberine was found to be 0.208%, 0.018%w/w, 0.636% and 0.203%w/w.

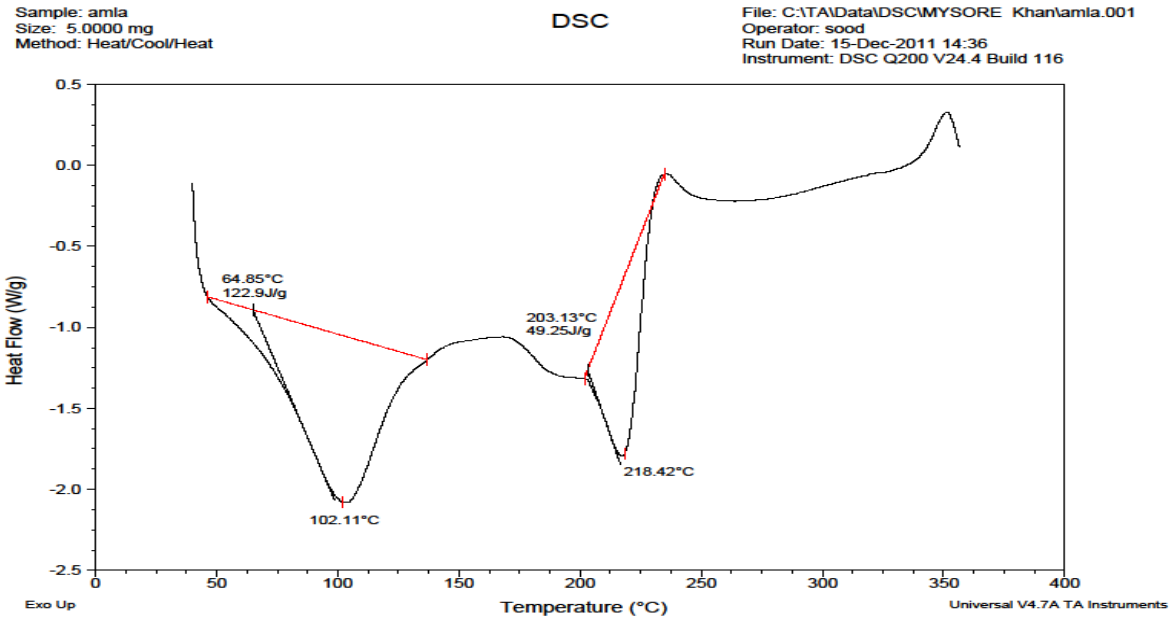

Fig. 3. Thermo gram of *E. officinalis*

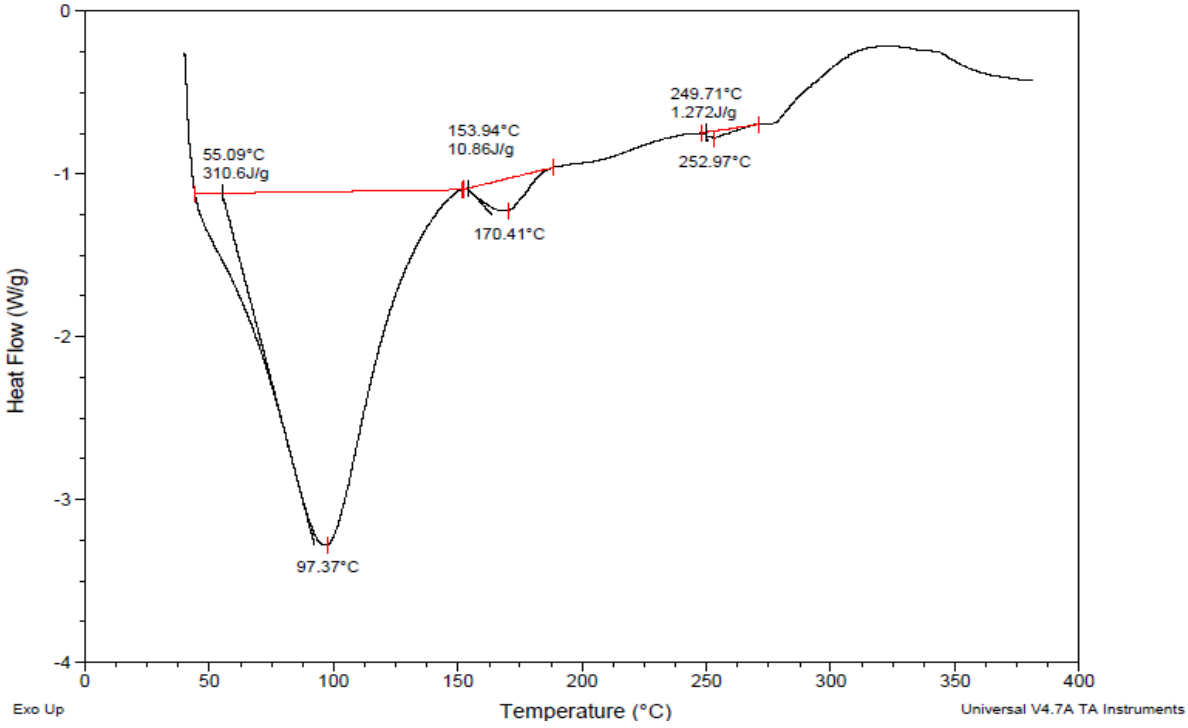

Fig. 4. Thermo gram of *Withania somnifera*

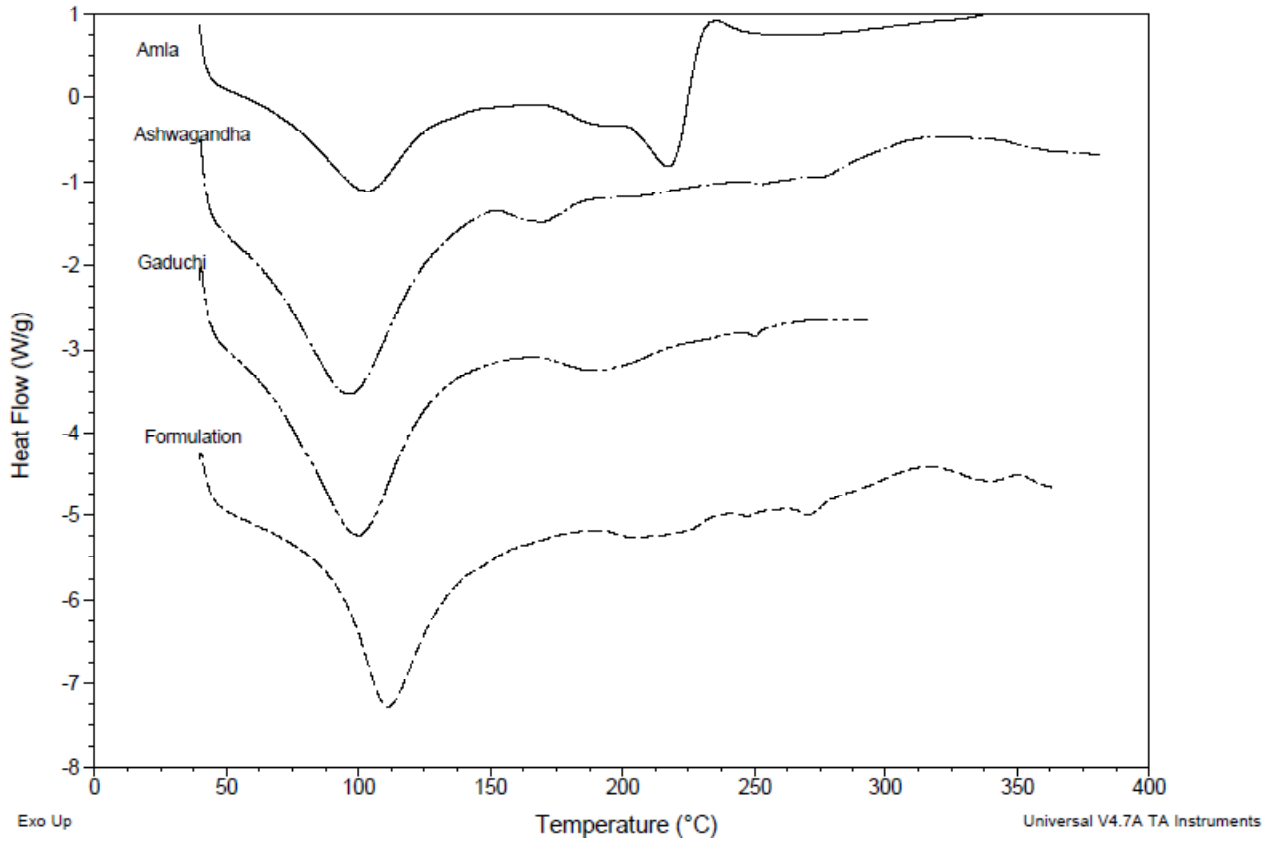

Fig. 5. Overlay of all thermo grams

The compounds like Withanolide A, Gallic acid, eugenol are proven to have immunomodulatory, antistress compounds and Rasayana properties and these were found out to be present in the formulation. Hence the formulation is said to be antistress herbal formulation.

CONCLUSION

The antistress formulation was formed with the help of four crude drugs viz. *Withania somnifera*, *Tinospora cordifolia*, *Emblica officinalis* and *Eugenia caryophyllus* and standardized as per the guidelines. Future prospects include the clinical trials of the finished product as the clinical efficacy is already proven in different animal studies.

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