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PREPARATION AND STANDARDIZATION OF DADRUNASHAK ARKA AND ITS ANTIFUNGAL ACTIVITY

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ABSTRACT

The skin is considered one of the primary sensory organs in Ayurveda. Skin diseases have been primarily classified in Ayurvedic literature under the category of Kushta, which means "that which causes disfiguration" in literature. There are many different etiological causes for dermatological disorders that are described in the Ayurvedic medical system i.e Physical, physiological, genetic, psychological, psychosocial, and Papakarma variables (bad deeds/psycho-social stress. In Ayurveda, skin conditions are categorized into two main types: Mahakushtha and Kshudrakushtha. Kshudrakushtha includes a specific type known as Dadrukushtha, which is often observed in clinical practice. The symptoms of Dadrukushtha closely resemble those of Tinea corporis, a condition recognized in modern science. In ancient Ayurvedic studies, our acharyas presented numerous Kalpanas to identify the major constituents of plant materials and herbs, which were then formulated into dosage forms, few of them are identified as basic types of Kalpana for e.g.: - kwatha, hima, phanta, sandhana. Arka Kalpana has its roots in Hima and Phanta Kalpana. One of them is Arka Kalpana (The distillation method) is used to extract the active ingredients from plant material or herbs. Dadrunashak Arka is a kalpa which is highly effective in Tinea Infection and is found in Ravankrut Arka Prakash (5/101), where its pharmaceutical preparation is easy in process as described in this book.

Dadrunashak Arka when prepared authentically and with appropriate precautions it shows remarkable results as an Antifungal drug against Microsporum Canis.

KEYWORDS: Dadru, Tinea corporis, kushtha, Fungal infection, Dadrunashak Arka.

1. INTRODUCTION

Skin disorders are often caused by lifestyle changes, insufficient exercise, and poor dietary choices. Other contributing factors include hygiene, emotional strain, and unhealthy eating patterns. This branch is dealt with formulations, therapeutic uses, and pharmaceutical products in Ayurveda. Determining dosage forms helps in this process. Arka is more pleasant form of Ayurvedic formulation is used instead of kalka, swarasa, kwath, etc. In studies of Arka Prakash, various types of dravyas are mentioned to undergo different procedures. This dosage form is effective and has better stability, compatibility, and patient compliance when administered in low dosages, being colorless. The potency, ease of absorption, and quick onset of action are more pronounced with Arka kalpana. As the present population is increasing day by day there's a need to study the simplified methodologies and procedures amplified in the composition of this formulation which could be easily acceptable and permissible in both scientific labs and at industrial levels. The detailed explanation of the pharmaceutical characteristics regarding this process is provided in Patras, Yantras, Agnis, and many other methods of preparation, depending on the consistency of dravyas. In the modern aspect, a distillation apparatus is used for the preparation of Arka.

The distillation method known as "arka kalpana" is used to extract the active ingredients in medications. It works well with medications that include more vaporous ingredients that evaporate when formed into kwath or choornas. Ravankrut Arka Prakash mentions DADRUNASHAK ARKA. A drug's arka is far more pleasant than other kinds of preparation and has a longer shelf life. It is also strongly potentiated. It is readily absorbed and can be used in lesser doses. India is a rapidly expanding country with fresh techniques emerging daily. As a result, there has been a rise in pollution, health misinformation, a hotter and more humid climate, a decline in living standards, and unclean conditions. This includes bacterial and fungal infections as the primary causes of skin disorders. One of the most prevalent fungi that grows in these circumstances is Tinea. Kushtha Chikitsa, an Ayurvedic text, covers the causes, symptoms, signs, and treatments of skin ailments. Tinea infection and Kshudra kushtha prakaar DADRU are quite similar. The components of Dadrunashka Arka, like Kushtha, Chakramarda, Haridra, Sarshapa, Laksha, Saindhava, and Amrasthi, possess

Krimighna and Kushthaghna properties. These ingredients are characterized by their Katu-Tikt-Kashaya (pungent-bitter-astringent taste), ushna virya (hot potency), and Katu Vipaak. Additionally, the dravyas are kaphahara and kleda shoshaka. As Dadru vyadhi is a Kaphaj Vyadhi, the combination of these medicinal substances, as described in Ravankruta Arka Prakash, helps in complete eradication of the disease. Moreover, the prepared 'Dadrunashak Arka' exhibits positive antifungal activity against Microsporum Canis species in comparison to Trichophyton Rubrum species. Dadrunashak Arka when prepared authentically and with appropriate precautions it shows remarkable results as an Antifungal drug against Microsporum Canis.

2. AIMS AND OBJECTIVES

2.1. Aim

To study the preparation and the analytical parameters of Dadrunashak Arka and evaluation of its Antifungal Activity.

2.2. Objectives of the study

- Preparation of Dadrunashak Arka from Ravankrut Arka Prakash by Distillation method.
- Study the Analytical parameters of Dadrunashak Arka.
- Perform Antifungal Activity of Dadrunashak Arka.

3. MATERALS AND METHODS

The study is conducted in following points,

- Collection of Raw Materials
- Preparing Dadrunashak Arka.
- Performing Analytical test of the final product.
- Analysing Antifungal activity of the final product.

Dadrunashak arka

कुष्ठं कृमिजदृघ्ननिशासैन्धवसर्षपाः। आम्रास्थिश्चैतदर्कौ वा लेपाद्दद्वं विनाशयेत्॥

(अ. प्र. ५/१०१)

3.1. Equipments

Distillation apparatus which consists of Heating mantle, still, condenser, rubber tube, receiver, stand.

3.2. Raw materials- in Coarse powder form:

1. Kushtha- 10 gm.
2. Laksha - 10 gm
3. Chakramarda- 10 gm.
4. Haridra – 10 gm
5. Saindhav lavan -10 gm
6. Sarshap - 10 gm
7. Amrasthi - 10 gm
8. Water -280 ml

Sr. No	Ingredients	Latin Name	Part Used	Quantity
1	Kushtha	Saussurea lappa	Mool (Root)	10gms
2	Laksha	Laccifera Lacca	Resin	10gms
3	Chakramarda	Cassia Tora	Seed powder	10gms
4	Amrasthi	Mangifera Indica.	Seed Powder	10gms
5	Haridra	Curcuma Longa	Rhizome	10gms
6	Sarshapa	Brassica Campestris	Seed and Seed oil	10gms
7	Saindhav lavana	Halite/ Rock Salt		10gms
8	Water			280ml

3.3. Procedure

In the present study, Dadrunashak Arka was prepared in distillation apparatus.

- Coarse powder of the drugs of Dadrunashak Arka is required for this Kalpana and hence coarse powder of all the ingredients of Dadrunashak Arka was purchased from local market and authentified.
- To get maximum quantity of water-soluble extract, 2 times water i.e. 280ml is required to soak the drugs for 24 hrs hence drugs become soft and Arka can be easily extracted out of it as written the text.
- After the absorption of whole amount of water in the choorna, again 2 times water was added as it was not sufficient to soak the powder properly and was kept in sunlight and under moonlight for 24 hrs.
- Next day it was transferred to distillation apparatus with condenser attached to it and closed properly.

- In distillation apparatus, 1/3 part of the flask should be filled with liquid to avoid frothing which may enter in condensing tube. If 3/4th part of the flask is filled with liquid, the final product is obtained in the form of vapour.
- Temperature control is an important factor in extraction of Arka. Boiling Point of water is 100° C hence it should be heated up to 100°C for the formation of vapour which is condensed further for the preparation of Arka. Temperature less than 100°C, is not enough to get Arka while temperature more than 100° C may burn the bottom of flask and Dagdha Paka of drug adds offensive/unpleasant odour to the final product.
- Initial part of Arka, contains less quantity of volatile extract. Similarly last part of Arka may contain residual particles hence initial as well as last part of vapour should not be collected to get better quality of Arka.
- After 30 - 35 minutes Arka vapours started draining out in the receiver.
- The first few drops were discarded.
- Arka was collected up to 1/3rd amount of water i.e. approx. 100ml.
- Again, heating was done remaining Arka was collected and stored in dark glassed air-tight bottle.

3.4. After completion of distillation process (Final product)

- The smell and taste of the Arka was same as that of the drug.
- Colour was white.
- Volatile oil was seen on the surface of liquid, which was yellowish to whitish in colour.

3.5. Precautions

1. Coarse powder is must for distillation.
2. Powder must be soaked for 18-24 hrs.
4. Two times water is not sufficient but 4 times water must be added for whole extraction.

4.1. Organoleptic character observations

Sr. no.	Character	Sample of dadrunashaka arka
1	Shabda	-
2	Sparsha (On touch)	Watery
3	Roopa (Appearance)	Yellow Opaque Liquid
4	Rasa (Taste)	Bitter
5	Gandha (Odour)	Faint

4.2. Analytical chemical tests

Sr. No.	Parameter	Sample of dadrunashaka arka
1	Specific gravity	1.0191gm/ml
2	pH	4.8
3	Refractive index	1.25
4	Boiling Point	102-degree Celsius
5	Volatile matter	0.13%

4.3. Observations of HPTLC test

Sr. No.	Frequency	Readings
1	254 nm	Prominent bands are seen at Rf 0.36,0.41 and 0.62
2	White light	Pink Intense bands are seen at Rf 0.26, 0.37, 0.44. Blue color bands are seen at 0.53.
3	366 nm	Intense bands 0.26 Light bands at Rf 0.39, 0.44.

4.4. Antimicrobial activity

Evaluation technique

Protocol: Antifungal property of test product by Well diffusion method

Test organism: Trichophyton Rubrum, Microsporum Canis

Under this heading antimicrobial study of three sample of Dadrunashak Arka was studied against Trichophyton Rubrum and Microsporun Canis.

Materials

A) Drugs: Sample of Dadrunashak Arka

B) Microorganisms

a) Trichophyton Rubrum

b) Microsporum Canis.

C) Equipment

1) Digital balance

2) Incubator

3) Autoclave

4) Inoculation hood

5) Hot air oven

6) Heating mantle

D) Glasswares

1) Petri dish

2) Conical flask
3) Test tube
4) Beaker
5) Funnel
6) Stirrer

E) Chemicals
1) Nutrient broth
2) Nutrient agar
3) Disttiled water
4) Surgical spirit

Method

Antimicrobial activity of Dadrunashak Arka was carried out by Agar well Diffusion method.

Principle

The microbial assay is based on the comparison at the inhibition of growth of microorganisms by measured conc. of the drug to be examined.

The antifungal activity was carried out by Agar well diffusion method.
The method depends on the diffusion of the drug from a cavity or well through the solidified agar layer of a petri dish to an extent, such that growth of the added microorganisms is prevented entirely in a circular area or zone around the cavity containing a solution of the drug.

The rate and degree of diffusion may be affected by concentration and type of salt, viscosity of solution, solubility, temperature, etc. It is the improper to compare the therapeutic value of the antimicrobial agent on the basis of size of the zone of inhibition as same excellent therapeutic agents diffuse poorly in agar vice versa.

Procedure

Preparation

All aspects of the Agar well diffusion method procedure is standardized to adhere to these standards. The media used in this testing was Sabouraud dextrose agar containing Chloramphenicol and cycloheximide at only 4mm deep was poured into either 100 mm or 150 mm petridishes. The pH level of agar must be between 7.2 and 7.4.

Incubation procedure

1) Using cork borer, a well of 8mm diameter was punched in the medium.
2) Using an aseptic technique, the fungal spores were harvested and standardised to approx. 10^{7} CFU/ ml. It was individually spread by a sterile swab evenly over the face of agar plate and then gently remove the excess liquid by gently pressing or rotating the swab against the tube.
3) Test material - Dadrunashak Arka in 100 ul quantity was then applied to each well. The plates were incubated at 28˚C for 5 – 7 days.
4) Zone of inhibition was measured by calibrated ruler.
5) Allow the plate to dry for approximately 5 minutes.
6) The plates were incubated at temperature of 28^{o}C.

Preparation of solution

A) Preparation of test solution: - Dadrunashak Arka sample was used directly as test solution.

B) Preparation of growth media: - Nutrient broth was used for the preparation of growth media.

Ingredient of nutrient broth gm/litre

1) Peptic digest of animal tissue - 5.00
2) Sodium chloride - 5.00
3) Beef extract - 1.50
4) Yeast extract - 1.50

Final pH (at 25^{0}C) is 7.4(+/-0.2).

Nutrient broth 13 gms was dissolved in 100 ml of distilled water and boiled for 15 mins. It was also allowed to cool. Around 100 ml was transferred to each conical flask and sterillised in autoclave at 15lbs pressure (i.e121^{0}C) for 20 min.

C) Preparation for inoculums: - A loop of organisms was emulsified in 100 ml sterile growth media under proper sterile condition and incubated for 72 hrs at 37^{0}C in incubator.

D) Preparation of Agar Plates: - 5ml of inoculums prepared was added to 45ml of flask containing nutrient agar at 37^{0}C.

- This was immediately powered into dry sterile petridish tothe depth of 5mm.

- The Petridish were placed on a leveled surface to ensure that layers of medium are of uniform thickness.
- Allow the plates to solidify at room temperature for 12hrs.
- Incubate the agar plates at 35^0C to check sterility.
- The surface of the agar layer was kept dry before use.
- With the help of sterile borer (Diameter 8 mm) cylinder were made in agar plates.
- Uniform volume (i.e.0.5ml) of test solutions were added to each cavity.
- After 30 mins agar plates were incubated at 37^0C for 72 hrs.
- Zone of inhibition was measured after 72 hrs.
- The diameter of the circular zone is the measurement of the zone of inhibition.

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or tip, and a volume (20–100 mL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. The agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

RESULTS

From the results, Microsporum Canis has shown remarkable results in Antifungal activity against Dadrunashak Arka.

Since the zone of inhibition

In vitro Antifungal Test of Dadrunashak Arka was carried out on following Microorganisms:

a) Trichophyton rubrum
b) Microsporum canis

Following Table shows Zone of Inhibition in Agar Media in mm of the Dadrunashak Arka:

Sr. No.	Test fungus	Sample of Dadrunashak Arka Zone of inhibition in mm
1	Microsporum Canis	26mm
2	Trichophyton Rubrum	Nil

Result

According to zone of Inhibition and criteria of assessment the results are as follows-

1. The zone of Inhibition was found to be 26 mm against Microsporum Canis Fungus, while it was found that the zone of inhibition to be zero mm against Trichophyton Rubrum.
2. Hence, we can say that this Kalpa, Dadrunashak Arka is definitely beneficial in Microsporum genus than in Trichophyton genera. Thus we can state that, in Invitro Antifungal Activity of Dadrunashaka Arka for above organisms shows good fungicidal activity against Microsporum Canis.

Population is increasing there's a need to study the simplified methodologies and procedures amplified in the composition of this formulation which could be easily acceptable and admissible both in scientific labs and at industrial levels. The pharmaceutical characteristics regards to this process have its detailed explanation in patras, yantras, agnis and many other methods of preparation depending upon the consistency of dravyas. This paper aims to highlight the importance of this practice, as in recent years it has been collectively understood the burning need for preparation of extracts which are to be used in the fields of cosmetics and treating therapeutic disorders. In considerations to modern aspect, a distillation apparatus is used for the preparation of A

DISCUSSION

Arka kalpana has the advantage of longer self-life period, transparency, easy administration, dose fixation, palatability and wide therapeutic range. In today's pharmaceutics the process of Arka Patana can be compared to distillation. Analytical tests of Dadrunashak Arka part exposes the hidden facts about the final product when it was critically analysed with help of physical and chemical parameters.

The Organoleptic Characters analysis reveals that the final product is transparent clear liquid. Its odour and taste are that of the drug used in it and watery in appearance.

All the ingredients of Dadrunashka Arka such as Kushtha, Chakramarda, Haridra, Sarshapa, Laksha, Saindhava and Amrasthi are krimighna and kushthagna.

All the drugs are Katu-tikta-kashaya rasatmaka, Ushna veerya, and katu vipaakatmak. Also, all the dravyas are kaphahara and kleda shoshaka. Since Dadru vyaadhi is kaphaj vyadhi the

combination of these drugs explained in Ravankruta Arka Prakash aids to eliminate the disease completely.

Also, the Antifungal Activity of ’Dadrunashak Arka’ prepared is also positive against Microsporum Canis species as compared to Trichophyton Rubrum species. Which hence proves that Ayurvedic formulations prepared and interpretated by our acharyas and explained in our granthas are potent and highy recommended in today’s era too

Pictures

Raw materials for preparing dadrunashak arka.

Soaking of raw materials in distilled water

Soaking the raw materials under sunlight for 24 hrs

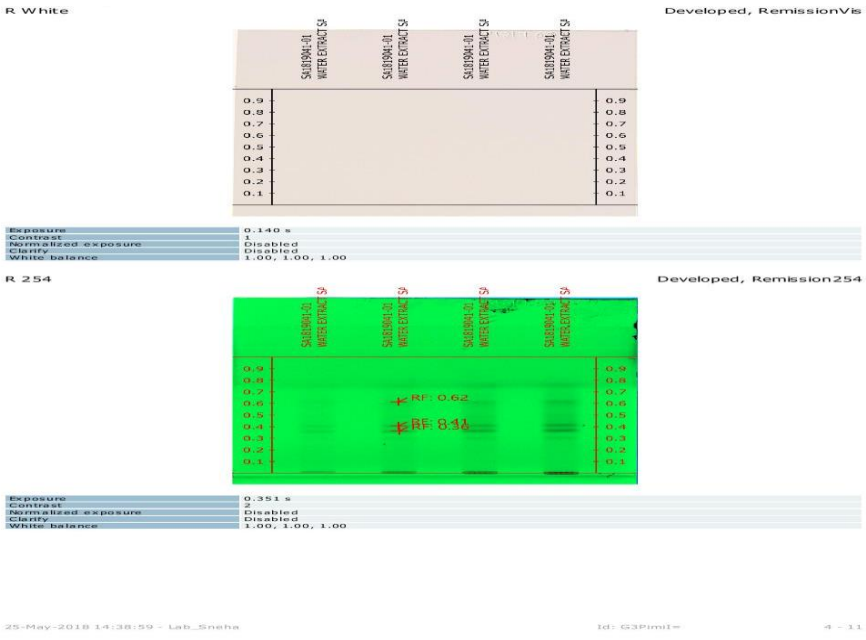
Preparation of dadrunashak arka using distillation apparatus

Temperature maintained at 95^0C

First drop apperared about 30- 35 mins of heating

Final product

RESULT OF HPTLC



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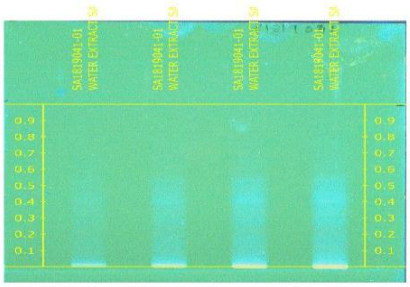

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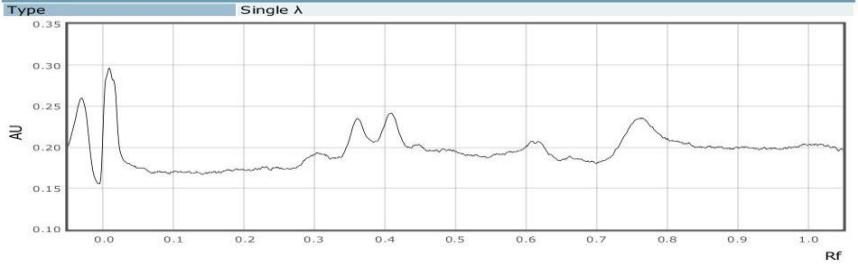

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Type	Single λ

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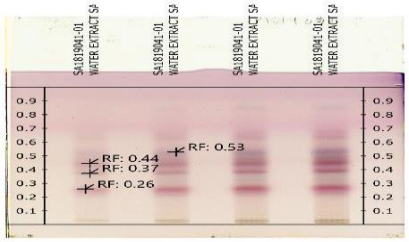
R White Derivatized, RemissionVis



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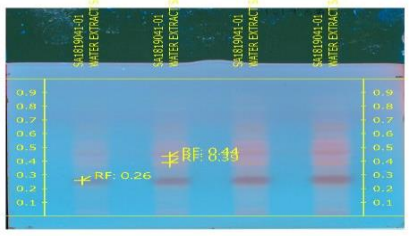

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Result of antifungal analysis of dadrunashak arka

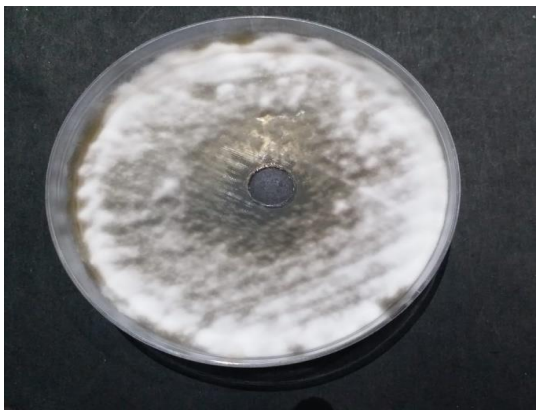

Image A

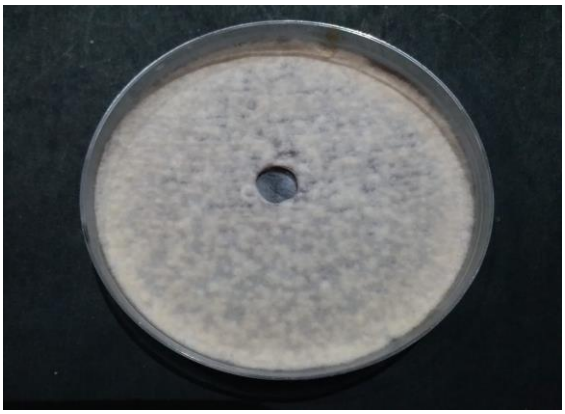

Image B

CONCLUSION

- Dadrunashak Arka is a kalpa which is highly effective in Tinea Infection and is found in Ravankrut Arka Prakash (5/101), where its pharmaceutical preparation is easy in process as described in this book.
- Glass apparatus for distillation process is convenient as compared to traditional Arka Patana yantra because ithas several draw backs such as difficulty in getting raw materials and Mritika to manufacture Yantra, easily breakable, leakage of Vapour, lack of experts having knowledge of manufacturing Yantra, etc. the preparation of Arka is preferred in distillation apparatus.
- Ideal Arka should have taste and odour of the arka should be more than that of the drug from which Arka Patan is done.
- When Arka is filled in different Patra, the colour of Arka should be similar to Shankha, Kundan and moon rays. Colour should not change if it is filled in Jirnasthi Mrutika Patra.
- It should be collected in Jirnasthi Mrutika Patra or kaachpatra (glassware) or pashan patra (stoneware).
- General Dose of Arka – 12-24 ml (AFI) and anupana should be Tambulbhakshana / Lavanga.
- All the analytical tests help in further standardzation of Dadrunashak Arka.
- Standards and specification which were present in olden days were sufficient to compete with time need but nowadays there is necessity to improve them to fulfil the norms of quality control and standardization, those which are must in nuclear era.
- The analytical data and HPTLC profiles evolved can be considered as viable parameters which will go a long way for prescribing dependable standards of these preparations.

- Invitro antifungal activity of Dadrunashaka Arka clearly shows that this kalpa has an excellent inhibitory action on Microsporum Canis fungi as compare to Trichophyton Rubrum.
- Agar well Diffusion method is convenient, cost-effective method for evaluation of Antifungal Activity of this kalpa.

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