

Development and Chemical Characterization of a Siddha Herbo-Mineral Preparation to Specific Eye Disease - *Thettran Kuzhambu* (TK)¹

Thiruvancheeswaran Soruban

Medical Officer, Siddha Teaching Hospital, Kaithady, Jaffna, Sri Lanka

Aravind Samy M.

Medical Practitioner, Aadhiran Siddha Hospital, Tirupattur, Tamil Nadu, India

Sudha Revathy S.

Assistant Professor, Department of Gunapadam, National Institute of Siddha,
Chennai, Tamil Nadu, India

Abstract

Background: Standardization of herbal formulations is an important factor to assess the quality, purity, safety, and efficacy of drugs based on the concentration of their active principles. It is very important to establish a system of standardization for every end product of medicine. In Siddha classical literature so many preparations are mentioned for Kan Noi (Eye Disease). Among these one of the preparation is Thettran Kuzhambu mentioned in the Siddha textbook of Balaramaiah, Siddha Maruthuva Nool Thirattu, in which Thettran Seed (*Strychnos potatorum*) is the main ingredient.

Objective: To prepare the Thettran Kuzhambu (TK) as per standard operating procedures mentioned in the classical text and to characterize it chemically using modern analytical techniques.

Materials and Methods: The prepared drug was chemically analyzed by Physico-chemical analysis, Microbiological test, HPTLC and ICP-OES were carried out as per WHO guidelines.

Results: The elemental analysis showed the presence of Mercury, Lead, Arsenic & cadmium below the limit of quantification. The HPTLC revealed the presence of Strychnine from plant material and microbiological load was found within permissible limits.

Conclusion: The present study reveals that the chemical standardization parameters of the drug Thettran Kuzhambu (TK) meets the prescribed standard limits and safe for further studies.

¹Address Author Correspondence to Thiruvancheeswaran Soruban at eleazarscaller@psu.edu.pa

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Keywords

Kan Noi, Eye Disease, Thettran Kuzhambu, Siddha, Herbo-Mineral Preparation.

Introduction:

Globally, eye diseases are considered as one of the major contributors of nonfatal disabling conditions in both high and low income countries. (1)(2) Eye diseases affect the quality of life. Geographical location, accessibility to facilities and socioeconomic status of an individual play a role in occurrence of eye diseases. (3) Many people are facing eye diseases like vision problem, allergic conjunctivitis, glaucoma, cataract, Night blindness, etc.(4)

The oldest traditional system of medicine is Siddha system and it had been coined and developed by Siddhars. Siddhars Nagamuni and Agasthiyar have described about 96 eye diseases in detail. (5) Many classical text books have described about eye diseases and preparations. These include Agasthiyar Nayana vidhi 500, Nagamuni Nayana vidhi 200, Sarabendrar Nayana roga sikichai, Pararasa segaram Nayana vidhi and Kandiraasa peril Nayana vidhi ennum ilangai singa mannan Nayana vidhi. (5),(6),(7) Among these one of the preparations is Thettran Kuzhambu (TK) mentioned in the Siddha textbook of Balaramaiah, Siddha Maruthuva Nool Thirattu, in which Thettran Seed (*Strychnos potatorum*) is the main ingredient. Indications of TK are Kanpadalam (Diseases of several coats over pupil), Kansivappu (Redness of eyes), Kannerichal (Burning sensation of eyes), Kanmangal (Blurred vision), and Kanpeelai thalluthal (Waxy deposits in corners of eyes). It can be applied using silver wire or sharpened and preserved rib of coconut leaves or on fingers twice a day. This produces a mild irritation. (8) This study was mainly conducted to prepare the TK according to SOP and also to evaluate the standardization parameters.

Objectives:

To prepare the Thettran Kuzhambu (TK) as per standard operating procedures mentioned in the classical text.

To characterize it chemically using modern analytical techniques

Methodology:

Collection of the drug

The raw drug Seed (*Strychnos potatorum*), *Cinnamomum camphora* and honey were purchased from authorized drug store in Parrys Corner, Chennai. The herbal drugs tender coconut water was purchased from Tambaram Market.

Ingredients

Seeds of *Strychnos potatorum* - 250g

Cinnamomum camphora - 0.175g

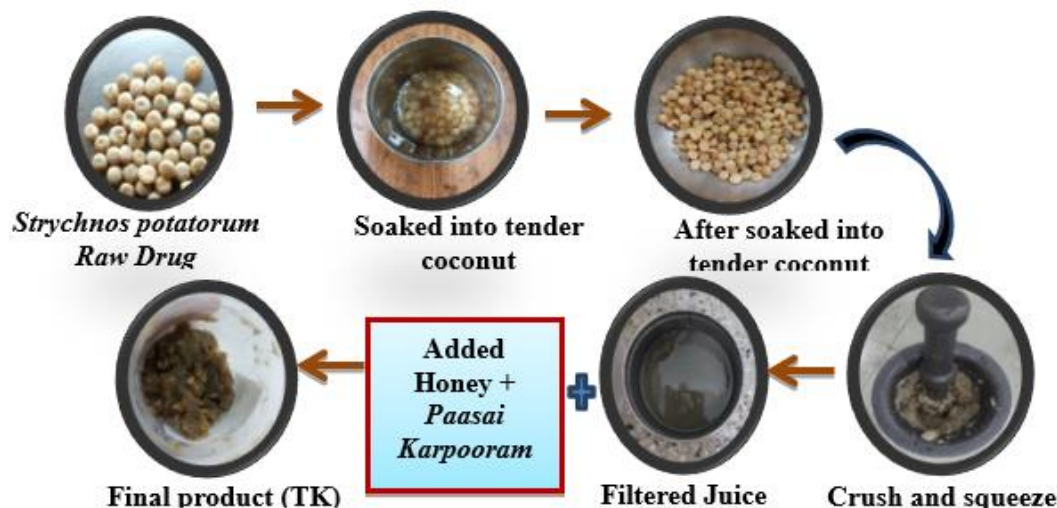
Honey - 17.5g

Tender coconut - Sufficient quantity

Identification and Authentication of drugs

Identification and Authentication of drugs. The raw materials were identified and authenticated by the experts of Gunapadam Department, National Institute of Siddha, Chennai 47.

Preparation process



Fg No: 1 Development steps of TK

Take the desired quantity of *Strychnos potatorum* seeds (250g) and immerse it in a filtered tender coconut water up to 15 days. The soaked seeds are then crushed with tender coconut water and then taken in a clean cloth and squeezed. After that wastage is eliminated. The obtained *Thetran kuzhambu* (70ml) is then mixed with honey (17.5ml) in the ratio of 4:1. It is then mixed with powdered *Pachai karpooram* (*Cinnamomum camphora*) approximately 2 grams for every one litre of the juice (0.175g). The mouth of the vessel is covered with thin muslin cloth and subjected to sunlight until the waxy consistency occurs. It is then transferred to the wide mouthed glass container and stored (30g). (Fg No: 1)

Standardization of drug

This was done as per the protocol for testing of Ayurveda, Siddha and Unani medicines by WHO guideline. Organoleptic Evaluation. (9)(10)

General Parameters

- **Colour:**

The TK was taken into watch glass and placed against white back ground in tube light. It was observed for its colour by naked eye.

- **Odour:**

The TK was smelled at two intervals. The time interval between them was about 2 minutes to nullify the effect of previous smelling.

Chemical Parameters

- **Determination of pH:**

10 gram of sample was weighed and mixed with 90 ml of distilled water. The mixture was stirred for three hours of time using rotary shaker. Then the pH was measured using pH meter.

- **HPTLC instrumentation and condition**

HPTLC CAMAG HPTLC Scanner (Model - Scanner III) and Aluminum coated silica gel 60F₂₅₄ TLC plate was used. Toluene: Ethyl acetate: Diethylamine mixture used in mobile phase and scanned wavelength were 250nm and 550nm. Injected sample volumes were 2 µl, 3 µl, 4 µl and 5µl. The Dragnodorff reagent used for derivatization. (11)(12)

Microbiological Tests

Preparation of Sample for Experimental Work (13)

Weighed 10 gm of the drug sample aseptically and dissolved in 10 ml of sterile water and made up to 100 ml with the sterile water. The insoluble drug product was suspended in 100 ml of buffered sodium chloride-peptone solution (pH 7.0).

Serial dilution of Sample (13)

A serial dilution is the dilution of a sample, in 10-fold dilutions. From the sample, 1 ml of the sample was added to 9 ml of sterile distilled water and mixed well. This dilution was denoted as 10⁻¹ dilution. From this dilution, one ml was taken from that mixture added to 9 ml, and designated as 10⁻² dilution. The same procedure was repeated up to 10⁻⁴

Isolation of Total Viable Aerobic Microbial Count Isolation of Bacteria by Plate Count Method (13)

In this test, the bacteria in the sample were made to grow as colonies, by inoculating a known volume of the sample into a solidifiable nutrient medium (Casein Soybean Digest agar or Nutrient agar medium)

in a Petri dish. The agar plate was prepared by mixing growth medium with agar and then sterilized by autoclaving. Once the agar was cooled to 45 °C, approximately 15 to 20 ml of medium was poured into a sterile Petri dish under aseptic condition and left to solidify for 15 minutes.

After solidification, each plate was smeared with 0.1 ml of sample from the dilution of 10^{-1} and 10^{-2} . After inoculations, all the plates were incubated at 37 °C for 24 hours. After incubation, the bacterial colonies were developed as visible to the naked eye and the number of colonies on a plate was counted using the Quebec Colony Counter. Plates with an average of 30 to 300 colonies of the target bacterium were selected for colony count. Because of the statistical problems, plates with lower than 30 colonies greater than 300 colonies were rejected.

Composition of Nutrient Agar Media (13)

Peptone : 5 gms, Sodium chloride :5 gms, Beef extract: 1.5 gms, Yeast extract: 1.5 gms, Agar: 5 gms, Distilled water: 1000 ml and pH: 7.4 + 0.2

Isolation of Fungi (13)

From each of the above-prepared samples, 0.1 ml of sample was transferred to Sabouraud Dextrose agar (SDA) prepared with Chloramphenicol. The plates were then incubated for 5 days at room temperature (20 to 25 °C). After incubation, the fungal colonies were observed and calculated.

Composition of Sabouraud Dextrose agar (SDA):

Dextrose: 40 gms, Peptone: 10 gms, Distilled water: 1000 ml and Agar: 15 gms

Heavy metals – ICP-OES (14)

- **Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis**

Assessment of heavy metallic constitution was made by Inductively coupled plasma optical emission spectrometer (Perkin-Elmer 5300 DV ICP-OES)

Result and Discussion

General Parameters

Table No: 1 Evaluated general parameters of TK

S. No	Parameters	Interpretation
1	Colour	Brown
2	Odour	Unpleasant

The Organoleptic characters of the TK were Brown colour, unpleasant smell and semi solid appearance.

Chemical Parameters

Table No: 2 Evaluated chemical parameters of TK

S. No	Parameters	Interpretation
1	pH	4.06
2	HPTLC	Strychnine present

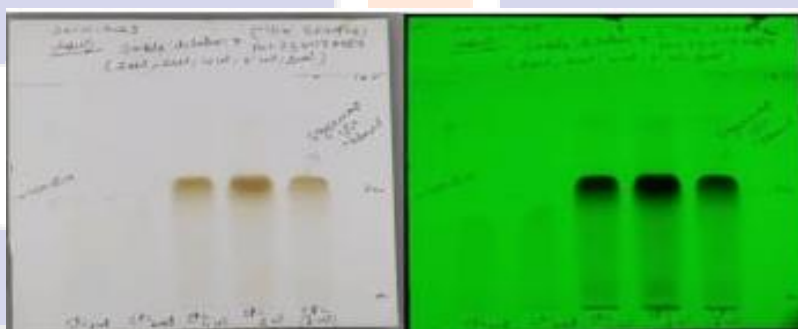
pH:

The pH of the TK is acidic. So it can induce lacrimation, ocular discomfort. But the reference textbook also mentioned as it can produce the symptoms of a burning sensation.

HPTLC:

Generally, HPTLC is chiefly used for phytochemical profiling of herbal drugs, validating its composition and also to overcome adulteration, etc. The HPTLC study carried out for TK chemical profiling will be helpful in the identification of bioactive compounds and markers, by comparing the R_f values of the compounds with the reference standards.

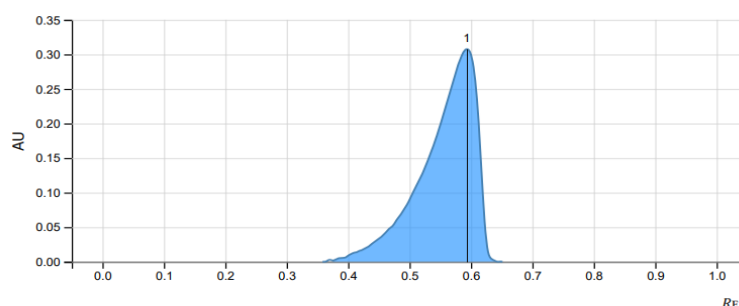
TLC plate under the visible light (After derivatization) and 254nm



Fg No : 2 Development TLC of TK

The spots were identified in the TLC plate under the visible light and 254nm at the loaded places of 3 μ l, 4 μ l and 5 μ l volume of samples. The spot of Strychnine is Observed in Sample at R_f (0. 0.57, 0.59, 0.58) (Faint Orange spot) and compared to that of standard sample (Fg No: 2)

Chromatogram of Sample 3 μ l TK at 550nm - Evaluation 1



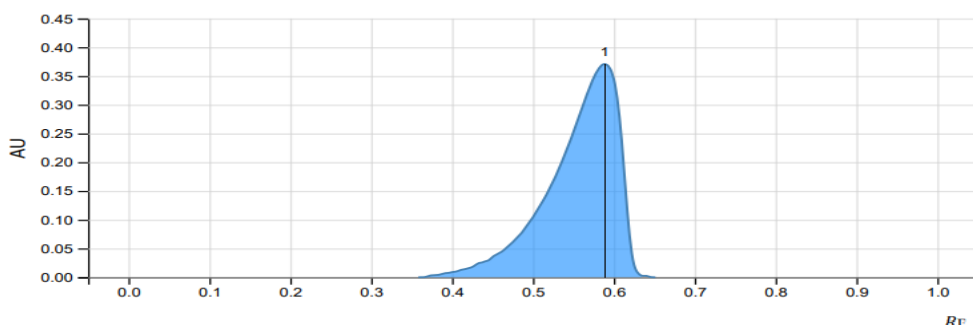
Fg No: 3 Chromatogram of 3 μ l TK at 550nm

Table No : 3 Interpretation of chromatogram of 3 μ l TK at 550nm

Peak No	Max R_f value	Max Height	Max %	Area	Area %
1	0.593	0.3075	100.0	0.02902	100.0

The interpretation of chromatogram of 3 μ l TK at 550nm (Fg No: 3) as tabulated in Table no: 3, it showed the presence of one compound which having max R_f value is 0.593 and area % is 100.

Chromatogram of Sample 4 μ l TK at 550nm - Evaluation 1



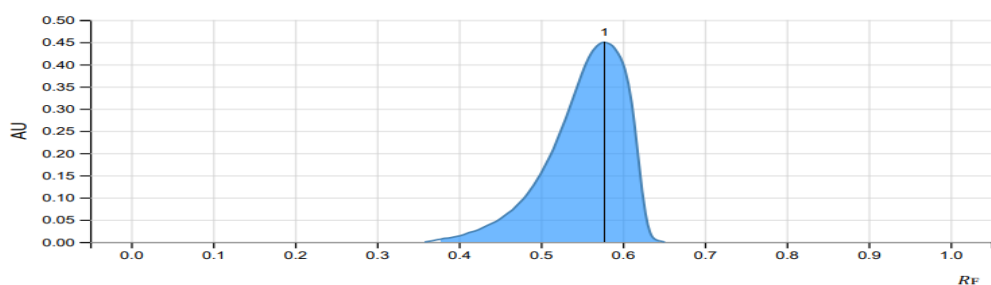
Fg No: 4 Chromatogram of 4 μ l TK at 550nm

Table No: 4 Interpretation of chromatogram of 5 μ l TK at 550nm

Peak No	Max R_f value	Max Height	Max %	Area	Area %
1	0.589	0.3707	100.0	0.03148	100.0

Fig No: 4 denoted the major phytochemical quantified in sample 5 μ l TK and indicated the presence of one compound, having max R_f value is 0.589 and area % is 100. (Table No : 4)

Chromatogram of Sample 5 μ l TK at 550nm - Evaluation 1



Fg No: 5 Chromatogram of 5 μ l TK at 550nm

Table No: 5 Interpretation of chromatogram of 5 μ l TK at 550nm

Peak No	Max R_f value	Max Height	Max %	Area	Area %
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1	0.578	0.4499	100.0	0.04823	100.0
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As shown in Table No 5, presence of one compound was identified having max R_f value is 0.578 and area % is 100 in the evaluation the chromatogram of 5 μ l TK at 550nm. (Fig No: 5)

Chromatogram of Sample 3 μ l TK at 550nm - Evaluation 2

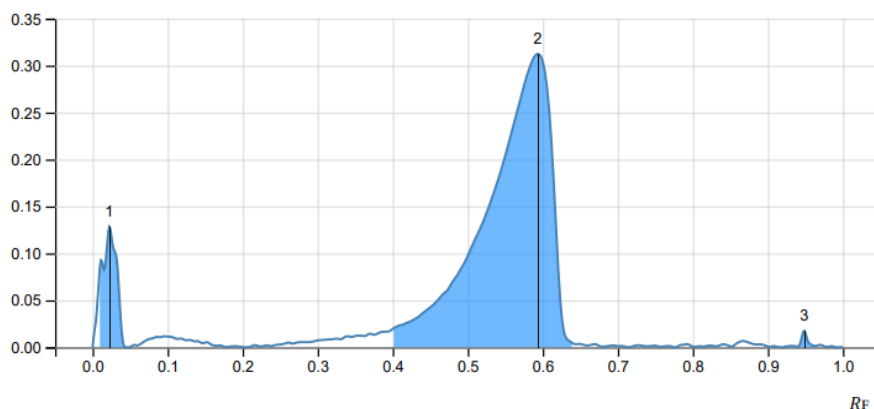


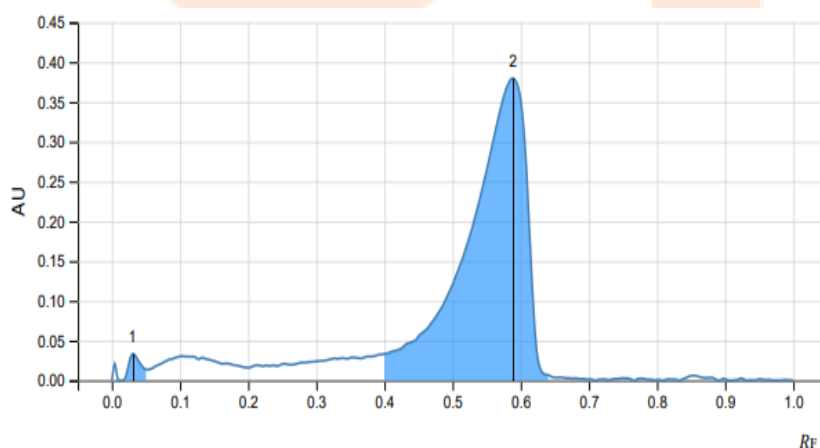
Fig No : 6 Chromatogram of 3 μ l TK at 550nm

Table No: 6 Interpretation of chromatogram of 3 μ l TK at 550nm

Peak No	Max R_f value	Max Height	Max %	Area	Area %
1	0.022	0.3139	41.89	0.00291	8.62
2	0.593	0.3061	40.84	0.03064	90.90
3	0.949	0.1294	17.27	0.00016	0.48

As shown in Table no 6, HPTLC finger printing analysis of the sample Interpretation the presence of three prominent peaks corresponds to the presence of three versatile phytochemicals (3 μ l TK) present within in the R_f value of the peaks ranges from 0.00 to 1.0. The identified, three compounds were having max R_f value between 0.022 and 0.949. Major compounds having area percentage and R_f value as 90.90% at 0.593 (Fig No: 6)

Chromatogram of Sample 4 μ l TK at 550nm - Evaluation 2



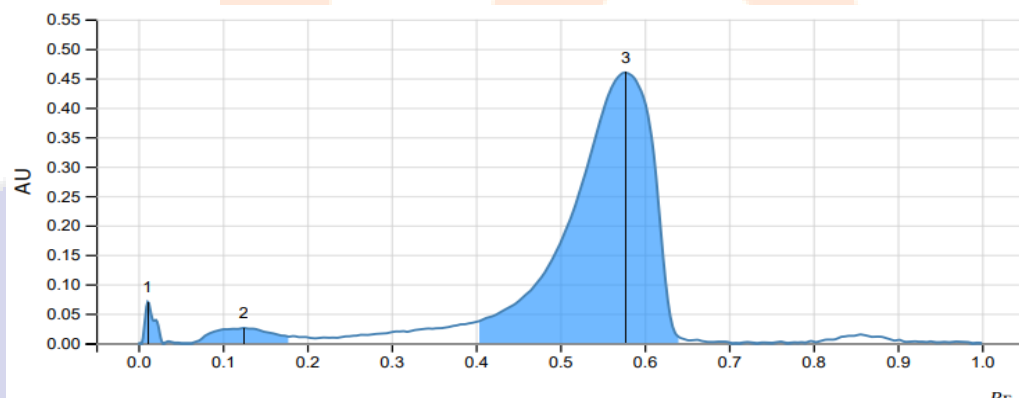
Fg No : 7 Chromatogram of 4 µl TK at 550nm

Table No: 7 Interpretation of chromatogram of 4 µl TK at 550nm

Peak No	Max Rf value	Max Height	Max %	Area	Area %
1	0.031	0.1791	32.69	0.00071	1.86
2	0.589	0.3689	67.31	0.03764	98.14

Chromatogram of 4 µl TK at 550nm reveals the presence of three prominent peaks corresponds to the presence of two versatile phytocomponents (4 µl TK) present within in the R_f value of the peaks ranges from 0.00 to 1.0, presence of two compounds were identified. having max R_f value between 0.031 and 0.589. Major compounds having area percentage and R_f value as 98.14% at 0.589 (Fig No: 7), (Table No: 7)

Chromatogram of Sample 5 µl TK at 550nm - Evaluation 2



Fg No: 8 Chromatogram of 5 µl TK at 550nm

Table No: 8 Interpretation of chromatogram of 5 µl TK at 550nm

Peak No	Max Rf value	Max Height	Max %	Area	Area %
1	0.011	0.3054	32.92	0.00092	1.69
2	0.125	0.1768	19.06	0.00208	3.81
3	0.578	0.4454	48.02	0.05144	94.50

Interpretation data of Chromatogram of 5 µl TK at 550nm results shown in Table No: 8, presence of three compounds were identified within in the R_f value of the peaks ranges from 0.00 to 1.0, having max R_f value between 0.011 and 0.578. Major compounds having area percentage and R_f value as 94.50% at 0.578. (Fig No: 8)

Microbiological Tests

Table No: 9 The microbiological load of TK

S. No	Parameters	Max Value	Interpretation
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1	Total viable aerobic count	1 x 100000	1300 cfu/gm
2	Total fungal count	1 x 1000	< 10 cfu/gm

According to the above table (Table No: 9) microbiological load was found within permissible limits. This result revealed TK does not have any microbial contamination and proved TK as a safe drug free from microbial contamination.

Heavy metals Tests

Table No: 10 The Heavy metalsof TK

S. No	Elements	Requirement	In Sample TK
1	Arsenic	3ppm	0.7
2	Mercury	1ppm	BLQ (LOQ 0.50)
3	Cadmium	0.3ppm	BLQ (LOQ 0.25)
4	Lead	10ppm	BLQ (LOQ 0.50)

Heavy metals analysis evaluated with help of the ICP - OES that result showed the presence of Mercury, Lead, Arsenic & cadmium below the limit of quantification (Table No: 10). Therefore, the TK is safe drug without any heavy metal toxicity.

Conclusion:

The HPTLC revealed the presence of Strychnine from plant material, microbiological load was found within permissible limits and elemental analysis showed the presence of Mercury, Lead, Arsenic & cadmium below the limit of quantification. So this study reveals that the chemical standardization parameters of the drug *Thettran Kuzhambu* (TK) meets the prescribed standard limits and safe for further studies.

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