

**RESEARCH ARTICLE****Isolation and characterization of bioactive molecule from *Lantana camara***Geetali S. Ingawale<sup>1</sup> and Anita S. Goswami-Giri<sup>2\*</sup><sup>1</sup>Shri JTT University, Vidyanagari, Churu Jhunjhunu Road, Chudela, Jhunjhunu, Rajasthan-333001, India.<sup>2</sup>Chemistry Research Laboratory, Department of Chemistry, B. N. Bandodkar College of Science, Chendani Bunder Road, Thane - 400 601 (MS) –India\*Corresponding Author E-mail: [anitagoswami@yahoo.com](mailto:anitagoswami@yahoo.com)**ABSTRACT:**

*Lantana camara* provides a huge amount of bioconstituents that is interest to exploit for natural products in medical field. Pentacyclic triterpenes/ lantadenes and other triterpenoids from *lantana* exhibited a wide array of pharmacological activities having potential for the development of antitumor therapeutic agents. Leaves of *Lantana* demonstrated its activity in aqueous, organic solvents and solvents ratio. Bioactivity of pentacyclic triterpenoids was exhibited with hydrovaccum distillation followed by gel chromatography and its enriched fractions of Lantadene analysed by spectroscopic, chromatographic methods and by surface morphology technique. This study may exploit for rational therapy of life.

**KEYWORDS:** *Lantana camara*; Lantadene; Pentacyclic triterpenoids ; bioactive molecule.**INTRODUCTION:**

*Lantana camara* Linn. is waste land notorious weed; belongs to the Verbenaceae family<sup>1</sup>. Different parts of this plant are used for medicinal and non-medicinal purposes including its complex are toxic to small ruminants. This effect has been associated with the types and relative amounts of some triterpene ester metabolites. However, *Lantana camara* it is rich in secondary metabolites possessing that are beneficial biological activities. In India, these plants are used on folk and traditional medicine system like antimicrobial, Fungicidal<sup>2,3</sup>, insecticidal and nematocidal activity including hepatotoxicing in animals.<sup>4</sup>etc. Verbascoside possesses antimicrobial<sup>5</sup> immunosuppressive and antitumor activities<sup>6</sup>. Plants also have ability to interfere with antibiotic resistance and its volatile constituents suppress the growth of staphylococcus aureus, pathogenic bacteria of respiratory system sources of metabolites<sup>7,8</sup>. Customization of *lantana* extracts as potential biocides have been suggested<sup>9</sup> as Lantane oil is dealing with skin itches as an antiseptic for wounds<sup>10</sup> and externally for leprosy and scabies<sup>11,12</sup>. Nevertheless, bearing antimicrobial activity but it is not promoting burn wound healing activity. The main chemical constituent; Lantadene is acts as promoting agent<sup>4,13</sup>. Due to its Polymorphism of chemical constituent and its polymorphic forms, differed in melting behavior.<sup>14</sup> The precise mechanism and nature of polymorphic forms have not yet been clear.

These constituents are reported to be influenced by genetic, geographical and seasonal factors as well as the developmental stages of the concerned plants<sup>15</sup> hence intensified research work needs to understand the chemical variation of lantadene in different solvent conditions.

**EXPERIMENTAL:****Plant material:**

The leaves of *Lantana camara* L were collected in January-February 2012 from Vidya Prasark Mandal's College campus, Thane (MS) India.

The collected material was air-dried at room temperature under shade for 8-10 days separately. The dried leaves were pulverized to powdery form using local mortar and pestle. The powdery form was then subjected to hydro distillation and stored at room temperature. Different parts of *Lantana camara* were subjected differently with alcoholic solvent methanol<sup>16</sup>.

**Part A: Extraction and Isolation of Bioactive molecule/ Lantadenes from *lantana* dried leaves using Solvent:**

*Lantana* leaf powder (100 g) and (500 ml) methanol was refluxed for 3hrs. Methanol was removed under vacuum (13-14 mm/Hg and Distillation temperature up to 58°C) to get concentrated residue which was suspended in distilled water. After filtration, the residue was suspended in a methanol–water (1:7) mixture and extracted with ethylacetate (2 X 25 mL) and with n-butanol ((2 X 25 mL)

The ethylacetate layer was concentrated under reduced pressure and chromatographed over silica gel column (30 g, 60–120 mesh) using chloroform and chloroform–methanol (9:1) as eluting solvent. The enriched fractions were rechromatographed on a silica gel column with n-Hexane by increasing amount of acetone. Different fractions were monitored with TLC, physical constant, UV, HPLC I.R. and qualitative analysis.

The above method was performed for stem powder, flower powder and black fruits separately.

**Part B. Extraction and Isolation of Bioactive molecule lantana dried leaves by green/Aqueous method:**

Lantana leaf powder (100 g) and (500 ml) distilled water was refluxed for 3hours. The treatment was given as per part A.

The pH of leaves extract was also observed throughout the study.

**Part C: Characterization of leaves enriched fraction eluted from solvent hydro distillation followed by gel chromatography:**

**Qualitative test for bioactive molecule /Pentacyclic triterpenoids:** Leaves active compound/ enriched fraction from silica gel column was treated with Chloroform and refluxed for 30 minutes, after cooling solution was treated with 3-4 drops of concentrated H<sub>2</sub>SO<sub>4</sub>.

**TLC:** Each enriched fractions from silica gel was monitored with TLC. The separation was achieved on TLC aluminum plates precoated with silica gel 60F254 using chloroform-methanol (95:5, v/v) as a mobile phase.

**UV:** Pure fractions wavelength was detected on UV 1800 Shimadzu spectrophotometer.

**HPLC:** Enriched fractions (2000 ppm) after re-chromatography was dissolved in minimum amount of solution in ACN: water (80: 20). Sample (5µl) was injected to HPLC to check the purity of elutant using C-18 column at 35°C. Stem powder, flower powder and black fruits enriched fractions also subjected to HPLC separately. Densitometry analysis of leaves enriched fractions was performed at 530 nm.

**SEM** of pure lantadene obtained from lantana leaves was carried out at Diya Laboratory, Thane,(MS).

**RESULT AND DISCUSSION:**

Extraction of different parts of *Lantana camara* Linn were carried out by reflux followed by column chromatography using different solvents. Organic solvents contributed exceptionally low yield of bioactive molecule showing very little difference in physicochemical properties of pentacyclic triterpenoids. It revealed that interferon of triumphing a pure form of Lantadene. Only ethylacetate does not able to extract pentacyclic triterpenoids /Lantadene completely while from the methanol–water mixture exhibited good yield. By selecting proper organic

solvents/methanol followed by fractional crystallization improved the yield of Lantadene. Leaves active compound/ enriched fraction from silica gel column produced dark red colored bulbous forms of Lantadene that are detected by qualitatively. (Figure 1) Flower and fruit extract surrounded low percentage of Lantadene which is confirmed by single pure peak on from HPLC (Figure 2). Bioactive molecule /Lantadene, was changes its physical property during processing. (Table1) In leaves, brownish shade to grayish while processing and in pure form, it reflects white in color.



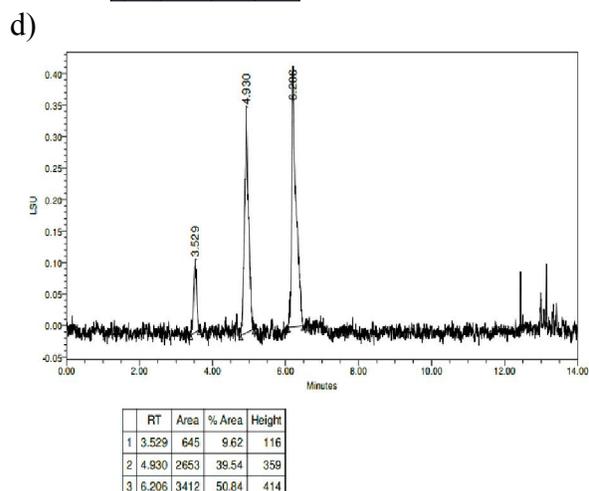
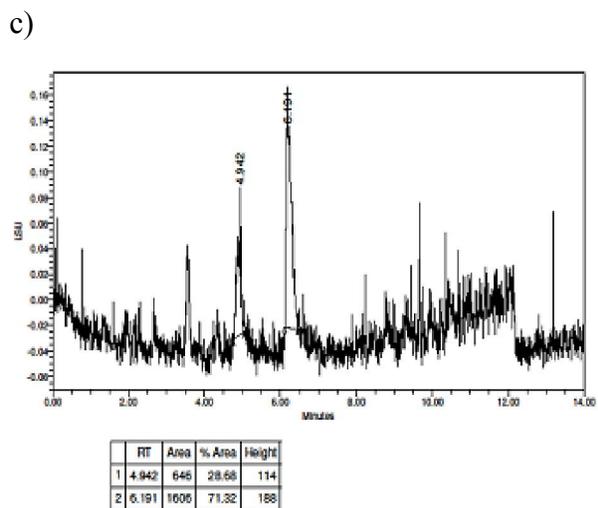
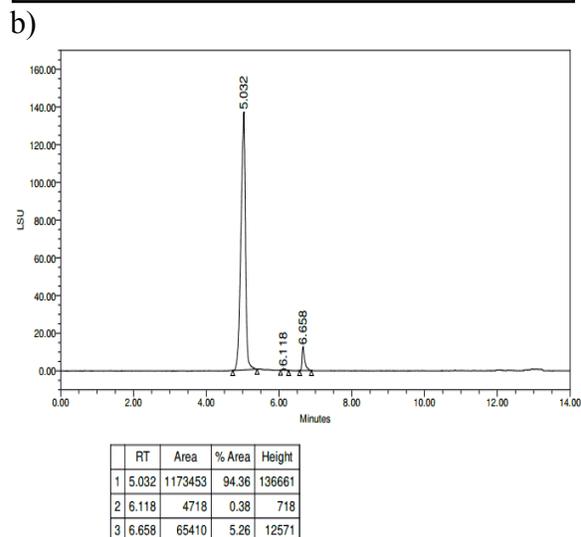
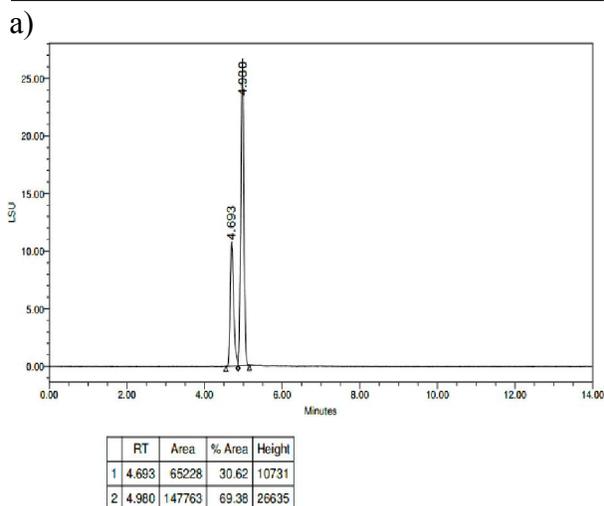
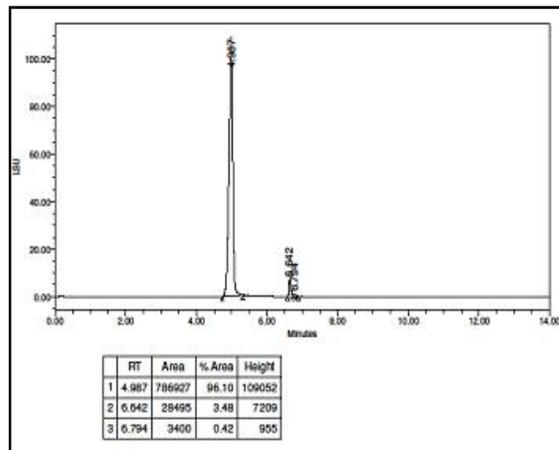
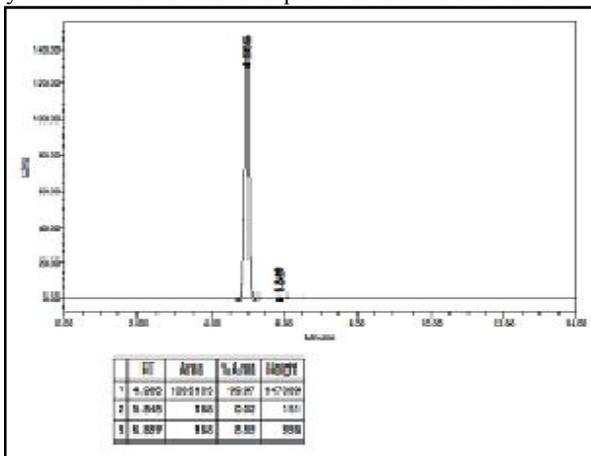
**Figure 1** Isolated bioactive molecule / pentacyclic triterpenoids . a) and b) Brownish residue of Leaves obtained solvent extraction and aqueous extraction under reduced pressure, c) Dark brown residue obtained from aqueous extract of *Lantana* leaves.

Lantadene (pentacyclic triterpenoids) 25 mgs was measured in leaves while 13 mgs was measured in stems. After repetitive study fruits and flowers crude extract does not elute pentacyclic rings. Hypothetically it may be bound in silica column if any. TLC of each sample was carried out separately using solvent system CHCl<sub>3</sub> –CH<sub>3</sub>OH ;( 9.8: 0.2) ratio.

In *lantana* flower powder analysis and ripened black fruits powder the ethylacetate layer was concentrated to get sticky mass. Sample was analyzed for HPLC and for detection of triterpenoids. Different enriched fractions pH observed is shown in table 2.

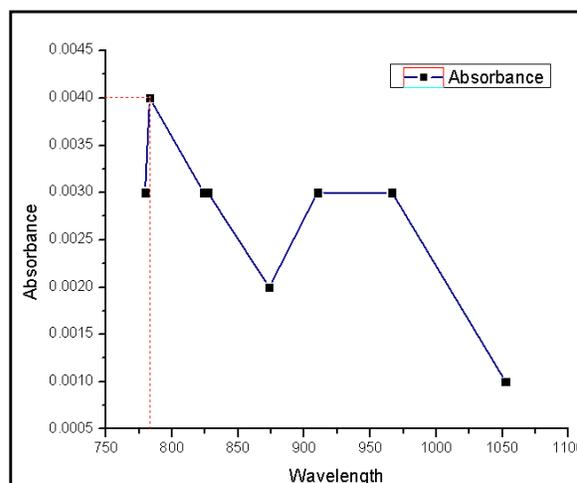
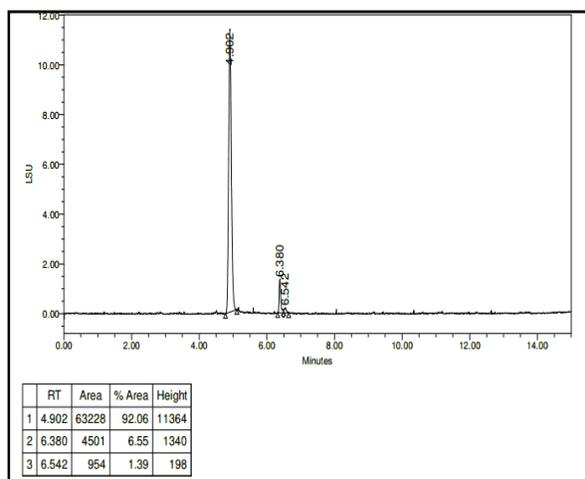
Melting point of Lantadene/ enriched fractions observed uncorrected however it start melting at 283°C. Aqueous reflux of *lantana* dried leaves, demonstrated that there was no separation of layers due to viscous mass. Colour of the layer was dark brownish liquid. After addition of excess

ethylacetate separation of layers it becomes very viscous indicating that, aqueous reflux method is not suitable for separation of triterpenoids and cannot compare to organic solvent reflux method.



e)

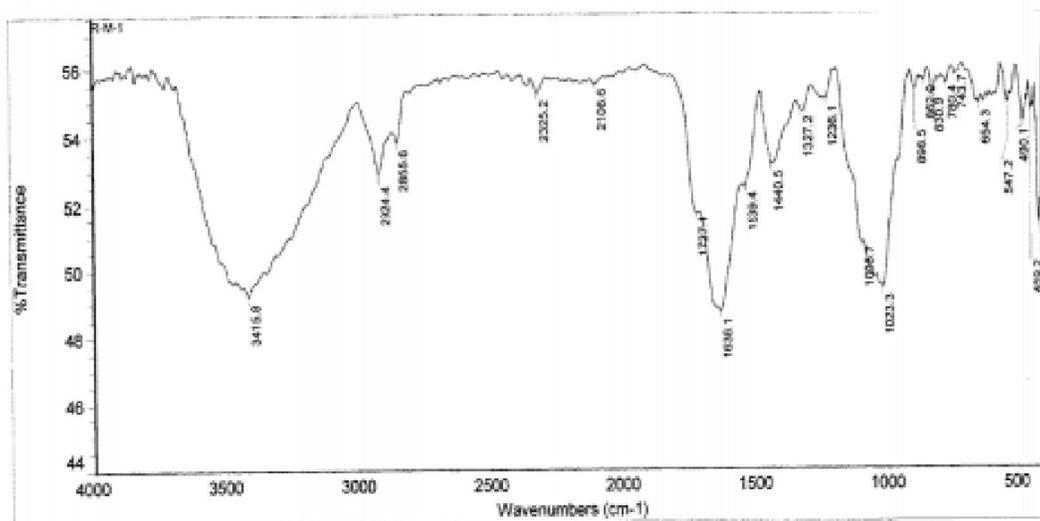
f)



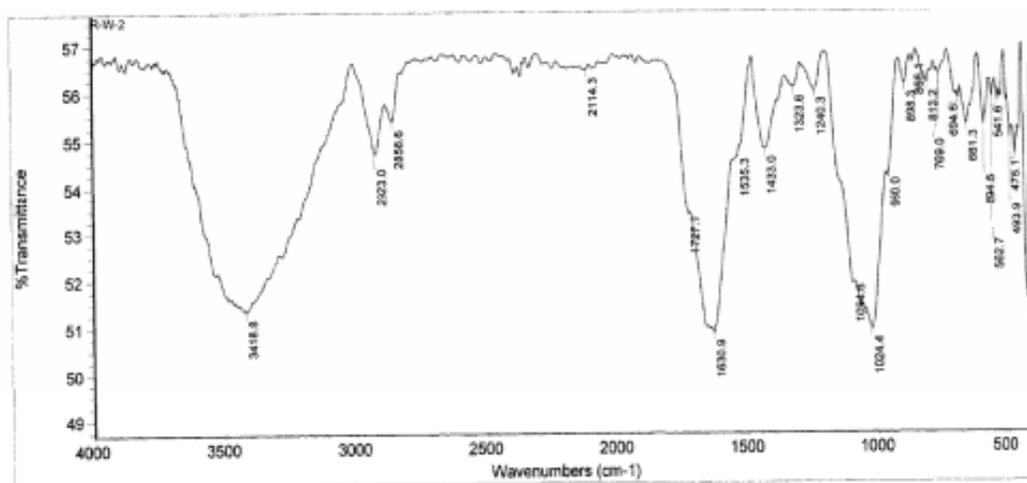
g)

h)

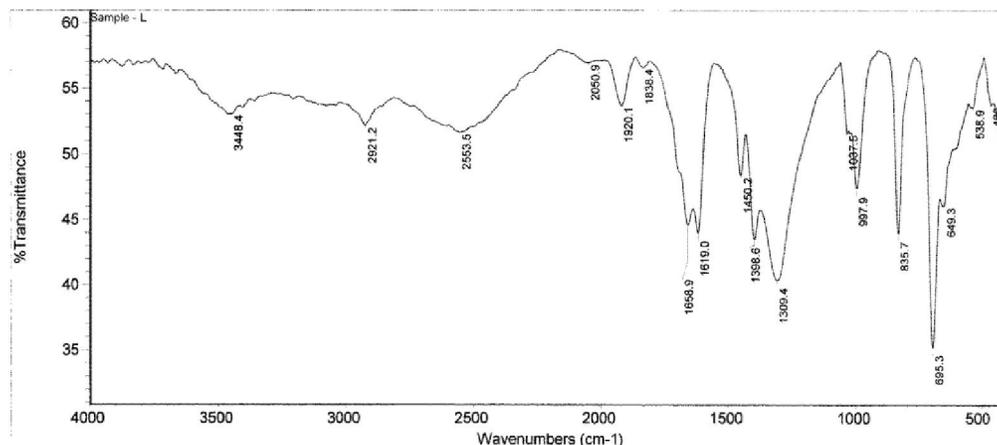
Figure 2 HPLC of showed single peak a) Standard b) aqueous extract (leaves) c) Ethylacetate extract (leaves) d) n-butanol extract(leaves) e) Ethylacetate extract(Flowers) f) Ethyl acetate Extract(Fruits) g) Ethyl acetate extracts (Stem).h) UV pure Lantadene



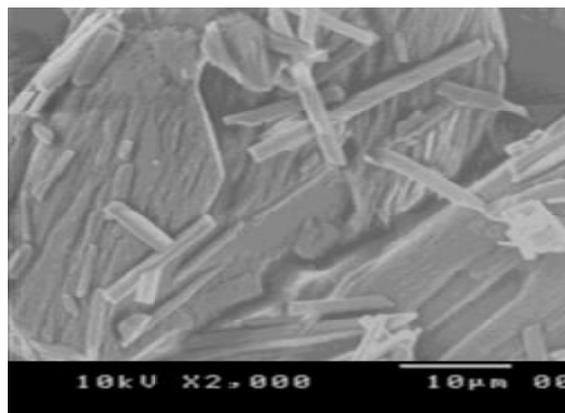
a)



b)



c)



d)

Figure 3 I.R. of leaves residue a) methanol reflux method b) aqueous reflux method, c) I.R. of pure lantadene d) SEM of pure lantadene

### Identification of Lantadene (pentacyclic triterpenoids)

#### Qualitative test:

Each extract qualitatively analyzed for triterpenoids nature. The appearance of red colour indicates the presence of triterpenoids the aerial part of lantana.

#### I.R. Spectrum:

3465  $\text{cm}^{-1}$  (-OH), 3076.5  $\text{cm}^{-1}$  (cyclic), 2925  $\text{cm}^{-1}$  (aliphatic C-H), 2536  $\text{cm}^{-1}$  (-COOH), 1925  $\text{cm}^{-1}$ , 1834  $\text{cm}^{-1}$  (Carbonyl group, 3 Keto), 1455  $\text{cm}^{-1}$  (aliphatic double bond), 1303  $\text{cm}^{-1}$  (O-C=O) linkage. (Figure 3a,b,c)

Noticeable differences in composition were observed with samples obtained from different locations in India. Considerable interest has been shown in the anti-inflammatory action of some Triterpenes having significant activity as inhibitors of human leucocyte elastase (HLE). Also participates in the destruction of elastin and plays a role in chronic disorders such as pulmonary emphysema, cystic fibrosis, hepatitis and rheumatic arthritis etc. Chemical composition of the whole plant, plant parts and essential oils are reported to be influenced by genetic, geographical, and seasonal factors as well as the developmental stages of the concerned plant, its

parts/tissues.<sup>17</sup> UV of isolated pure lantadene from leaves showed in figure 2 (h). Its densitometric analysis was performed at 530 nm showed pure peak, are highly stable as evident from their absorption spectra which are consistent throughout the analysis of each fractions.

#### SEM:

A pentacyclic triterpenoid compound from *lantana* (*Lantana camara*) leaves has been obtained in two polymorphic forms I and II. Form I had white, fluffy, and rod-shaped uniform crystals (figure 3d) Form II particles were irregular, shining, and polyhedral. The two forms differed in melting behavior. The powder x-ray diffraction of form I showed sharp peaks whereas form II did not contain distinct peaks (unpublished work).

Laboratory and field evaluations have been conducted on the efficacy of aqueous and organic Extracts, and topical application of ground powders from roots, leaves, stems and flowers on several insect pests.<sup>18</sup> similarly, aqueous extracts exhibited strong antifeedant effect on *Plutella xylostella* (Lepidoptera) but were not repellent. This property indicates that each extract may identify novel compounds having their own characteristic.

## CONCLUSION:

Aerial parts of *Lantana camara* Linn produced extremely low yield of pentacyclic triterpenoids in organic solvents extraction by hydrodistillation followed by column chromatography while it is not measurable in aqueous solvent. Physicochemical properties of pentacyclic triterpenoids revealed that, difficulty in getting pure form of Lantadene. Ethylacetate own sort out whole extract Lantadene completely from the methanol–Water mixture. Flower and fruit extract contain low percentage of Lantadene. Qualitative test, spectrophotometric and chromatographic analysis gives depth of purity of subject. But study suggests selection of proper organic solvents and fractional crystallization methods may improve yield of Lantadene.

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