

Original Article

A COMPARATIVE PHARMACOGNOSTICAL EVALUATION OF THREE BOTANICAL SOURCE PLANTS OF JIVANTI

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Received: 04 Oct 2017 Revised and Accepted: 21 Dec 2017

ABSTRACT

Objective: This study was undertaken to carry out a comparative pharmacognostical evaluation of three botanical source plants used in the name of classical Ayurvedic drug Jivanti.

Methods: Leaves of three source plants of jivanti belonging to Asclepiadaceae family i.e. *Leptadenia reticulata* (Retz.), *Holostemma ada-kodien* Schult. and *Wattakaka volubilis* (Linn. f.) Stapf were evaluated for morphological and microscopical characters including quantitative microscopy, surface study, powder microscopy and histochemical studies.

Results: Morphologically all the three species showed some similar characters like simple, opposite leaves with reticulate venation. The shape of the leaves were ovate to oblong in *L. reticulata* and *H. ada-kodien* whereas *W. volubilis* was having broadly ovate or suborbicular leaves. Multicellular glandular warty trichomes, rosette crystals of calcium oxalate, lactiferous cells were observed in all the three species whereas prismatic crystals were also present in *W. volubilis*. Palisade ratio and stomatal index were higher in *H. ada-kodien* followed by *L. reticulata*. Characteristic differences in the organoleptic characters like colour, taste, touch were observed in individual powder samples. Test for lignin, calcium oxalate crystal, starch grain and tannin showed a positive result in all the three samples.

Conclusion: The present study reports specific pharmacognostical characteristics for the identification and differentiation of each botanical source plant. The observed results can also serve as a reference for any further investigations.

Keywords: *Holostemma ada-kodien* Schult, Jivanti, *Leptadenia reticulata* (Retz.), Pharmacognosy, *Wattakaka volubilis* (Linn. f.) Stapf.

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DOI: <http://dx.doi.org/10.22159/ijpps.2018v10i2.22915>

INTRODUCTION

Jivanti is one of the important medicinal herbs having immense medicinal excellence described in almost all classical texts of Āyurveda. Classical texts of Āyurveda highlighted jivanti as the best vegetable (śreṣṭha śāka) to be consumed for maintaining the good health [1]. But, the botanical source of jivanti is in the state of controversy. Though *Leptadenia reticulata* (Retz) is accepted as an official botanical source [2], various herbs are used under the name of jivanti in the different parts of the country, namely *Wattakaka volubilis*, *Holostemma ada-kodien* Schult, *Dendrobium macraei* Lindl etc. [3]. Use of these botanicals may be due to different regional names, wide availability in the natural source as well as in local market etc.

Evaluation of plant materials through their pharmacognostical characters plays a very significant role in the area of authentication of correct plant source. In standardization of a crude drug, macroscopic and microscopic evaluation is the primary step. The original and basic approach towards pharmacognosy includes the study of morphological characters, cell structures, organization and study of tissue system, which holds an important role in the identification and the better understanding of the correct species of the plant and also helps to differentiate between closely resembled species. According to world health organization (WHO), botanical standard investigations like epidermal cells, stomatal index etc are mandatory for the diagnosis of the herbal crude drug [4, 5].

Review of literature shows that only a few preliminary works have been carried out regarding their pharmacognostical study. A detailed and comparative pharmacognostical evaluation of different source plants of jivanti is still lacking. Hence, in the present study, three source plants of jivanti i.e. *Leptadenia reticulata* (Retz.), *Holostemma ada-kodien* Schult. and *Wattakaka volubilis* (Linn. f.) Stapf were studied in detail to establish their differential botanical characters.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals used in the study i.e. Phloroglucinol, Conc. HCL, Iodine and Ferric chloride are of analytical grade and purchased from Sigma-Aldrich, India.

Collection and preservation of the sample

All the plant materials were identified on the basis of their morphological characters with the help of local plant collectors, botanical texts and flora [6, 7]. The collected plant materials were authenticated by an expert taxonomist. Leaves of *Leptadenia reticulata* (Retz.) were collected from Sasoi botanical garden and Gujarat Ayurved University (GAU) botanical garden. *Holostemma ada-kodien* Schult. and *Wattakaka volubilis* (Linn. f.) Stapf. were collected from Rakha khatia forest area of Jamnagar, Gujarat during September 2015. All the plant materials were collected following standard collection practices. Colour photographs were taken during collection and herbarium specimen of each sample were prepared following standard guidelines. Sample specimen of each drug was deposited to pharmacognosy museum, G. A. U., Jamnagar. (Specimen No-PHM 6202-*Leptadenia reticulata* (Retz.), PHM 6207-*Holostemma ada-kodien* Schult., PHM 6208-*Wattakaka volubilis* (Linn. f.) Stapf., PHM 6209-*Dendrobium macraei* Lindl) for future references.

Morphological study

The morphological study includes size, shape, apex, margin, venation, base, petiole, surface, the color of leaves of *L. reticulata*, *H. ada-kodien* and *W. volubilis*.

Microscopical study

Detailed microscopic characters were studied by taking freehand thin transverse section. Sections were stained with Phloroglucinol

and Hydrochloric acid to notice the lignified elements like fibers, vessels etc [8, 9]. Photographs of the section were taken with the help of Canon digital camera attached to Zeiss microscope.

Quantitative microscopy

Quantitative microscopy was carried out to determine epidermal cell number, stomatal number, stomatal index and size of the stomata [10].

Powder microscopy

Dried leaf powder of all the three species was studied following standard procedures[11]. The microphotographs were taken by using Carl zeiss trinocular microscope.

Histochemical test

To confirm the presence and absence of the chemical constituents the material were subjected to various tests. The histo-chemical tests were carried out according to the standard guidelines of practical pharmacognosy [12].

RESULTS AND DISCUSSION

Morphology

Morphologically all the three plants are distinct in their appearance and can be easily identified. Leaves of all the three species belonging to Asclepiadaceae family, are simple and opposite. Stipules are very small or absent in *L. reticulata* whereas *H. ada-kodien* and *W. volubilis* were ex-stipulated. Comparatively, the petiole is larger in *W. volubilis* and *H. ada-kodien*. The shape of the leaves was ovate to oblong in *L. reticulata* and *H. ada-kodien* whereas *W. volubilis* is having broadly ovate or suborbicular leaves. Leaves of all the three species belonging to Asclepiadaceae family showed reticulate venation with 4-6 pairs of nerves. The texture of leaves was hirtellous in *L. reticulata* whereas in *H. ada-kodien* and *W. volubilis*, leaves were glabrous above and pubescent beneath. The shape of the apex was acuminate in *W. volubilis*, cuspidate in both *L. reticulata* and *H. ada-kodien*. Leaves of *L. reticulata* were having obtuse or sub cordate base whereas the shape of the base was deeply cordate and rounded in *H. ada-kodien* and *W. volubilis* respectively (table 1).

Table 1: Comparative morphology of leaves of three source plants of jivanti

| S. No. | Parameter | Results | | |
|--------|----------------|--|---|---|
| | | <i>L. reticulata</i> | <i>H. ada-kodien</i> | <i>W. volubilis</i> |
| 1. | Type | Simple | Simple | Simple |
| 2. | Phylotaxy | Opposite | Opposite | Opposite |
| 3. | Stipules | Very small or absent | Exstipulate | Exstipulate |
| 4. | Petiole | 1.1-2.1 cm | 2.8-4.5 cm | 3.2-5.8 cm |
| 5. | Shape and size | Ovate to oblong, 5-4.7 cm | Oblong-ovate, 8.2-11×3-5.6 cm, upper leaves somewhat triangular | Broadly ovate or suborbicular, 6.5-14/4.5-11 cm |
| 6. | Venation | 6 pairs of nerves with reticulate venation | 4-5pairs of nerves with reticulate venation | Reticulate venation |
| 7. | Texture | Hirtellous above, pubescent beneath | Glabrous above, thinly pubescent beneath | Glabrous above, less softly pubescent beneath |
| 8. | Apex | Cuspidate | Cuspidate | Acuminate |
| 9. | Base | Obtuse or subcordate | Deeply cordate, 5-7 glands at the base of midrib | Rounded, few small glands just above the petiole. |

Table 2: Comparative microscopical characters of three source plants of jivanti

| Parameter | | Results | | |
|-----------------|---------------------------------------|--|---|---|
| | | <i>L. reticulata</i> | <i>H. ada-kodien</i> | <i>W. volubilis</i> |
| Petiole | Shape | Circular | Deeply concave in upper side | Circular |
| | Epidermis | Single layered | Single layered | Single layered |
| | Epidermal cells | Thin walled and very small | Thin walled and small | Thin walled and small |
| | Cuticle | Thin | Thick | Thin |
| | Trichomes | Multicellular glandular | Multicellular warty | Multicellular, warty |
| | Hypodermis | 3-4 layers of circular to oval collenchyma cells with angular thickenings | 2-3 layers of collenchyma cells | 3-4 layers of collenchyma cells. |
| | Cortex | Thin-walled circular to oval parenchymatous cells with distinct intercellular spaces. | 5-6 layers of parenchymatous cells. | Thin-walled circular to oval parenchymatous cells with large intercellular spaces. |
| Crystals | | Several prismatic crystals are present | Rosette and prismatic crystals of calcium oxalate | Several prismatic, rosette crystals are present |
| | Vascular bundle | Crescentric bicollateral vascular bundle, xylem is located in the centre followed by phloem on both sides. | Arranged in crescentic shape in the middle, separated by wide areas of ground tissue. | Bicollateral vascular bundle, protoxylem facing towards center and metaxylem towards the epidermis. |
| | | | | |
| Mid rib | Shape | Strongly convex | Broadly semi circular | Strongly convex |
| | Upper epidermis | Single layer | Single layer | Single layer |
| | Epidermal cell | Barrel-shaped | Oval to rectangular | Barrel-shaped |
| | Trichomes | Multicellular, glandular warty trichomes | Multicellular warty | Multicellular and glandular trichomes |
| | Cuticle | Thick | Moderate | Thick |
| | Hypodermis | 1-2 layers of compactly arranged palisade parenchyma with oil globules and rich in chloroplast | Single layered, elongated barrel shaped palisade parenchyma cells with numerous chloroplasts. | 1-2 layers of palisade parenchyma cells with chlorophyll pigments and oil globules |
| | Crystals | Rosette crystals of calcium oxalate | Prismatic crystals | Prismatic and rosette crystal |
| Vascular bundle | Open and bicollateral vascular bundle | Open and bicollateral vascular bundle | Centrally located bicollateral vascular bundle. | |

Microscopy

Among the three source plants of jivanti, studied for their microscopic characters, the common characters of Asclepiadaceae family and some individual species characters were observed. All these microscopical characters can be used for identification of the species and to differentiate each other. T S of *H ada-kodien* was deeply concave in upper side whereas in *W volubilis* and *L reticulata* T S was circular in shape. Epidermis was single layered in all the three species. Cuticle layers are thick in *H ada-kodien*, *L reticulata* and *W volubilis* composed of thin cuticle. Specific characters of Asclepiadaceae family like crescentric bicollateral vascular bundles, multicellular glandular warty trichomes, prismatic and rosette crystals of calcium oxalate were seen in all the three species.

Transverse section of midrib of *L reticulata*, *H ada-kodien* and *W volubilis* was studied and compared for their identical and differential characters. T S of mid rib was strongly convex in *L reticulata* and *W volubilis*, broadly semicircular in *H ada-kodien*. Epidermis was single layered and covered with cuticle in all the three species. Epidermal cells were barrel-shaped in *L reticulata* and *W volubilis*, oval to rectangular shaped in *H ada-kodien*. Multicellular glandular warty trichomes, rosette crystals of calcium oxalate, lactiferous cells were observed in all the three species whereas prismatic crystals were also present in *W volubilis*. All the three species showed centrally located, open, bicollateral vascular bundle.

Surface study

Surface study plays an important role in drug identification. The importance of epidermal characters, in general, is widely recognized in taxonomic considerations and in many cases, these are

successfully used in the identification of taxa at genus as well as species levels [13]. Similarly, studies in stomata have a great taxonomic as well as pharmacognostic value in the proper identification of medicinal plants [11]. In the present study, stomata were rarely distributed in the upper epidermis in *W volubilis* whereas stomata were absent in *L reticulata* and *H Ada-kodien*. Some of the trichomes and cicatrix were also observed in *W volubilis*. The lower epidermis composed of paracytic stomata, trichomes and cicatrix in *L reticulata* and *H ada-kodien*, paracytic and anisocytic stomata and in *W volubilis*.

Quantitative microscopy

All the three source plants composed of a paracytic type of stomata. Size of lactiferous cells, warty trichomes and epidermal cells were larger in *H ada-kodien* compared to other species. Size of the palisade cells was almost similar in *L reticulata* and *W volubilis* whereas it was much smaller in *H ada-kodien*. Cuticle layer was thicker in *L reticulata*. Length and the surface measurement of xylem fibres were more in *L reticulata*. Palisade ratio and stomatal index were higher in *H ada-kodien* followed by *L reticulata* (table 3).

Powder microscopy

Characteristic differences in the organoleptic characters like colour, taste, touch were observed in individual powder samples. Different organoleptic characters observed during the study are presented in table 4.

Histochemical study

Test for lignin, calcium oxalate crystal, starch grain and tannin showed a positive result in all the three samples. (Table 5).

Table 3: Comparative quantitative microscopy of leaves of three source drugs of jivanti

| S. No. | Parameter | Results | | |
|--------|---|-----------------------------|-----------------------------|--------------------------------------|
| | | <i>L reticulata</i> W and A | <i>H ada-kodien</i> schult. | <i>W volubilis</i> (Linn. f.) stapf. |
| 1. | Type of stomata | Paracytic | Paracytic | Paracytic |
| 2. | Size of stomata (Length X width) | 0.60X0.30 µm | 0.75X0.63 µm | 0.8 X 0.6 µm |
| 3. | Lactiferous cavity (surface) | 366.60 µm ² | 848.68 µm ² | 498.83 µm ² |
| 4. | Xylem measurement from proto to metaxylem | 119.08 µm | 102.95 µm | 106.11 µm |
| 5. | Xylem surface measurement | 861.25 µm ² | 770.42 µm ² | 391.71 µm ² |
| 6. | Rosette crystals | 329.31 µm ² | 436.78 µm ² | 568.12 µm ² |
| 7. | Warty trichome | 3735.54 µm ² | 7320.29 µm ² | 3845.65 µm ² |
| 8. | Palisade cell measurement | 1040.41 µm | 406.65 µm | 1080.48 µm ² |
| 9. | Epidermal cell measurement | 217.11 µm ² | 419.99 µm ² | 212.64 µm ² |
| 10. | Cuticle layer measures | 32.42 µm | 25.13 µm | 18.55 µm |
| 11. | Stomatal index | 25 | 27 | 3 |
| 12. | Palisade ratio | 3 | 4 | 2.5 |

Table 4: Organoleptic characters of the three source drug of jivanti

| Organoleptic characters | <i>Leptadenia reticulata</i> W and A | <i>Holostemma ada-kodien</i> schult | <i>Wattakaka volubilis</i> L. f |
|-------------------------|--------------------------------------|-------------------------------------|---------------------------------|
| Colour | Light green | Dark green | Light green |
| Taste | Slightly bitter | Sweet and bitter | Slightly sweet and bitter |
| Touch | Smooth | Smooth | Smooth |
| Odour | Characteristic | Characteristic | Characteristic |

Diagnostic character like paracytic stomata, rosette crystals of calcium oxalate, and lactiferous cells were observed in all the three species. *L reticulata* and *H ada-kodien* showed multicellular warty trichomes whereas multicellular and glandular trichomes were observed in *Wattakaka volubilis*.

Table 5: Histochemical study of three botanical source drug of jivanti

| Reagents | Test for | Observation | <i>L. reticulata</i> | <i>H. ada-kodien</i> | <i>W. volubilis</i> |
|--------------------------|-------------------------|------------------------|----------------------|----------------------|---------------------|
| Phloroglucinol+Conc HCL | Lignin | Red colouration | ++ | ++ | ++ |
| Phloroglucinol+Conc HCL | Calcium oxalate crystal | Dissolved | ++ | ++ | ++ |
| Iodine | Starch | Blue | ++ | ++ | ++ |
| Ferric chloride solution | Tannin | Blue-black colouration | ++ | ++ | ++ |

'++' Present



A. *L. reticulata*



B. *H. ada-kodein*



C. *W. volubilis*

Fig. 1: Morphological characters of three source plants of jivanti



A. *L. reticulata*



B. *H. ada-kodein*



C. *W. volubilis*

Fig. 2: Measurement of leaves



A. *L. reticulata*



B. *H. ada-kodein*

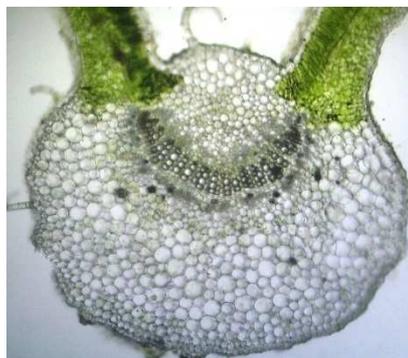


C. *W. volubilis*

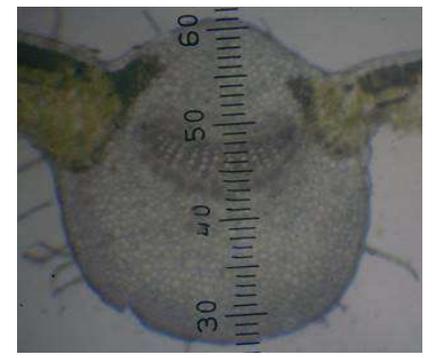
Fig. 3: T S of petiole



A. *L. reticulata*

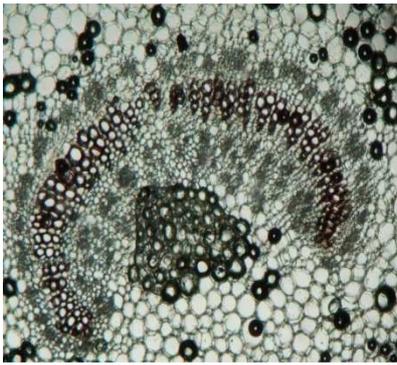


B. *H. ada-kodein*

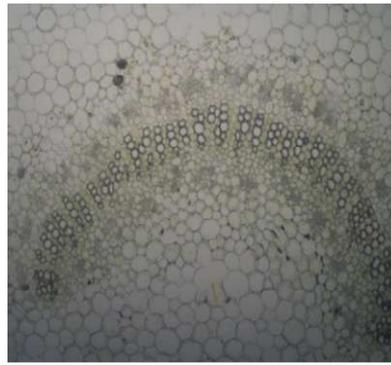


C. *W. volubilis*

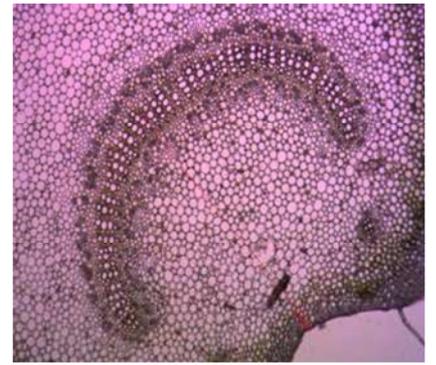
Fig. 4: T S of mid rib



A. *L. reticulata*

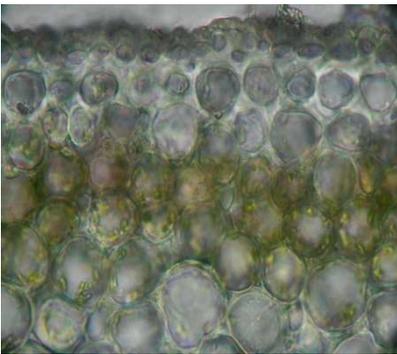


B. *H. ada-kodein*

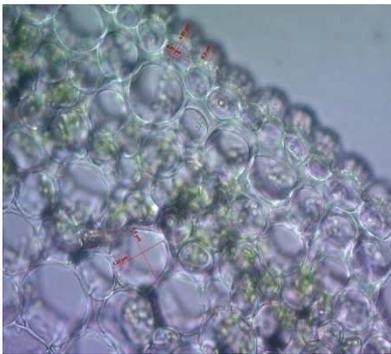


C. *W. volubilis*

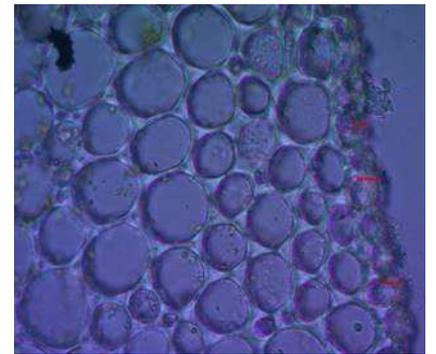
Fig. 5: T S of petiole showing vascular bundle



A. *L. reticulata*



B. *H. ada-kodein*



C. *W. volubilis*

Fig. 6: T S of petiole showing epidermis and hypodermis



A. *L. reticulata*

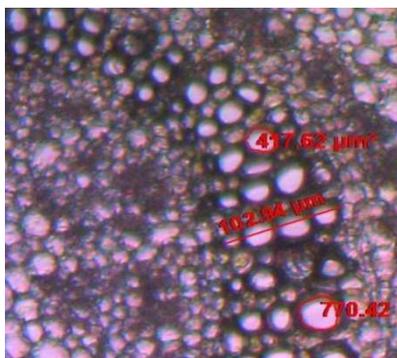


B. *H. ada-kodein*



C. *W. volubilis*

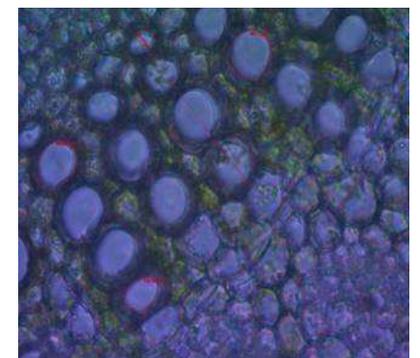
Fig. 7: T S of petiole showing trichomes



A. *L. reticulata*



B. *H. ada-kodein*



C. *W. volubilis*

Fig. 8: T S of petiole showing xylem fibres

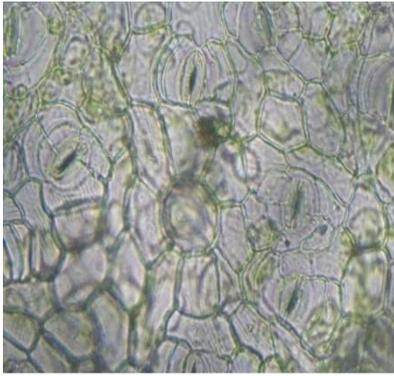
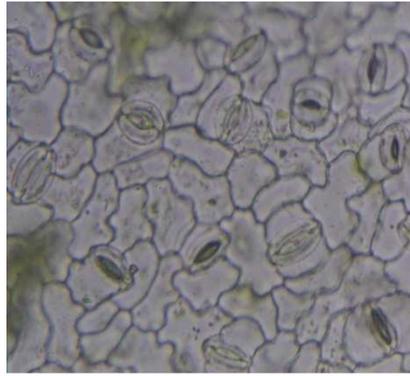
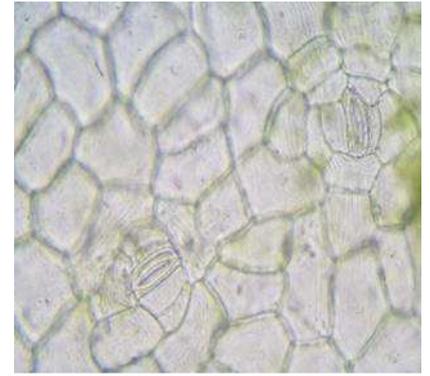
A. *L. reticulata*B. *H. ada-kodein*C. *W. volubilis*

Fig. 9: Surface study

CONCLUSION

The observed macroscopical and microscopical characters are useful for the identification and differentiation of closely related species used in the name of jivanti. The results of comparative quantitative microscopy are reported for the first time. These observations are specific to the species and can be considered as the diagnostic characters of the individual sample.

AUTHORS CONTRIBUTIONS

Dr Raghavendra Naik conceptualized, designed, carried out the work and drafted the article. Dr Rabinarayan Acharya, conceptualized, designed, monitored the work and edited the article. Dr Harisha C R supervised the experimental study and edited the manuscript.

CONFLICT OF INTERESTS

The authors do not have any conflict of interest to declare.

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