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Original Article

Morpho-Anatomical Studies on *Secamone emetica* (Retz.) R. Br. ex Sm.-An Endemic Medicinal Plant

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ABSTRACT

Background: *Secamone emetica* belongs to the family Apocynaceae (globally known to possess a broad spectrum of therapeutic properties). In folkloric medicine this plant is used to treat headache, leucorrhoea, fever, dysentery, etc. Paliyan tribes of Sirumalai Hills use this plant to treat nervous disorders. **Objectives:** The objective of the present study is to evaluate the morpho-anatomical features of *Secamone emetica* (Apocynaceae), an endemic medicinal plant of South India. **Materials and Methods:** Stem sections of *Secamone emetica* were taken with the help of a Rotary Microtome. Powder microscopic analysis was done using standard procedures. **Results:** Intraxylary phloem, mucilage cells, prismatic crystals, laticifers, xylem with solitary vessels, longer vessel elements, vasicentric tracheids and uniseriate hetero cellular medullary rays were observed. Powder microscopic studies revealed the presence of cork cells, bordered pitted vessels, fibro sclereid, and prismatic crystals. **Conclusion:** The present findings will be helpful in the correct identification and standardization of *Secamone emetica*.

Keywords: Intraxylary phloem, Laticifers, Prismatic crystals, *Secamone emetica*, Stem.

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INTRODUCTION

Since antiquity, a huge pool of herbal medicinal wisdom has emanated anecdotally in the form of myths, incantations, and religious belief systems.[1] Years of obsession with modern medicinal systems people have adopted the "back to nature" concept because of the unpleasing side effects of modern medicine.[2,3] Hence demonstrating the anatomical characteristics of the plant will help in the standardization and proper identification of plant samples. The genus *Secamone* belonging to the family Apocynaceae (Subfamily: Secamonoideae) is a pantropical genus of about 162 species distributed in Africa, Asia, and Australia. *Secamone emetica* is a native plant of South India mostly found at 1200 m elevation, from Andhra Pradesh to Tamil Nadu.[4] It is a climber and grows primarily in the seasonally dry tropical Biome. Traditionally this plant is used to cure various disorders including headache, leucorrhoea, fever, dysentery, etc. Paliyan tribes of Sirumalai Hills use this plant to treat nervous disorders.[5] Milky latex possesses several secondary metabolites rich in hydrocarbon content.[6] Sini *et al.* (2010) reported the antioxidant property of fruits and leaves.[7] Previous reports on other species of *Secamone* revealed that *Secamone afzelli* is reported to have antioxidant and anti-inflammatory properties.[8-10] Further, it alleviates prostatic hyperplasia exogenously.[11] Methanolic extract of leaves showed strong presence of flavonoid and sixteen different phenolic acids and insecticidal activity.[12-14] Mensah, *et al.* (2006) confirmed the folkloric uses of *Secamone afzelli* by reporting the anti-microbial and anti-oxidant properties.[15] In addition coumarins were identified and purified by methanolic extract of *Secamone afzelli* leaves and stem.[16,17] *Secamone volubilis* is traditionally used in treating diarrhea, febrifuge, diabetes, anti-hypertensive and hernia it also has good anti-microbial activity against Staphylococcus aureus and radical scavenging activity.[18,19] *Secamone africana* is reported with anti-plasmodial, purgative and anti-helminthic activity.[20]

Despite the genus *Secamone* being reported to have potential benefits and the plant *Secamone emetica* having been also reported in folkloric medicine, the plant has not yet been explored scientifically. Upon reviewing the literature, no scientific evidence supports its validation. Owing to this our study was focused on elucidating the macro-microscopical characters of*Secamone emetica* stem which is essential for the correct identification and authentication of this plant.

MATERIALS AND METHODS

Plant Material

The test plant *Secamone emetica* (Retz.) R.Br. ex Sm**.** was collected from Palamalai, Mettur, Erode District, Tamil Nadu, India and authenticated at Botanical Survey of India (BSI), Southern Circle, Coimbatore, India and the herbarium of the voucher specimen

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number BSI/SRC/5/23/2022/Tech.528 was deposited at the PG and Research Department of Botany, Vellalar College for Women, Erode, Tamil Nadu, India. The collected living materials were fixed in fixative FAA for anatomical studies. The Stems were dried, ground into a powder, sifted through a 40-mesh sieve and preserved in an airtight container for later use.

Chemicals and Instruments

Rotary Microtome, Compound microscope, Axiolab 5trinocular microscope, glass slides, cover slips, watch glass and other common glassware were the apparatus and instruments used for the study. Photographs were taken using Nikon D-5600 Digital camera and Zeiss Axiocam208 color digital camera. All the reagents used in the present study were procured from HiMedia Laboratories, Mumbai.

Macroscopic Study

The morphological characters, such as colour, odour, taste, shape and size etc. of *Secamone emetica* (Apocynaceae) were analysed botanically and photographed under original environment.[21]

Microscopic Study

Preparation of Specimens

Fresh samples of *Secamone emetica* were preserved in FAA fixative (Formalin, 5 mL, Glacial acetic acid, 5 mL, and 70% Ethyl Alcohol, 90 mL) for over 48 hr, following the schedule outlined by Sass (1940).[22] The specimens were infiltrated gradually by adding Tertiary Butyl Alcohol (TBA) to paraffin wax (melting point 58-60°C) until the TBA solution was supersaturated. The specimens were encased into paraffin blocks.

Sectioning and Staining

The specimens embedded in paraffin were cut into thin transverse sections using a Rotary Microtome, with each section measuring 10-12 micrometers in thickness. The customary method was employed to remove the wax from the sections. To enhance the comprehension of some anatomical structures within the sections, they were stained with Toluidine blue and Safranin.[23]

Photomicrographs

After staining, all permanent slides were dehydrated using Ethanol and Xylol and then mounted in DPX for preservation. Microscopic descriptions of tissues were complemented with micrographs wherever applicable. Photographs at various magnifications were captured using a Nikon D-5600 Digital camera and a Zeiss Axiocam208 color digital camera. Normal observations were conducted using a bright field, for the crystal studies polarized light was used due to their birefringent properties, making them appear bright against a dark background. The descriptive terminology for anatomical features was referred from a standard anatomy book.[24]

Powder Microscopy

A little portion of the powdered sample had been cleared with a saturated solution of chloral hydrate and placed on a microscope slide along with a droplet of 50% glycerol. Characters were examined in a bright field and polarised light using an Axiolab-5 trinocular microscope equipped with an Axiocam208 colour digital camera. Photomicrographs of distinctive features were captured and documented.[22-25]

RESULTS

Macroscopical Study

Wiry, much branched, climbing shrub, stem slightly woody at the base, sparsely pubescent when young, glabrous at age, latex milky-white. Leaves chartaceous, linear-lanceolate or ovate-lanceolate, 3.3-5.7×0.7-1 cm, glabrous, apex acutely apiculate, base acute, margin slightly revolute, lateral veins 3-8 pairs, petiole 1-6 mm. Flowers in axillary dichotomous cymes, peduncle 8 mm; calyx 5-lobed, 1cm diam.; corolla campanulate, 4 mm long, yellowish, lobes recurved; pollinia erect; corona single, staminal, 5-lobed, small, adnate to stamen, beaked; ovary 7 mm. Follicles paired, cylindric, divaricate,7.5×1 cm, apex beaked with blunt tip, slightly winged; seeds oblong, 7×3 mm (Figure 1).[21]

Microscopical Study

T.S. of Young Stem

T.S. of young stem exhibits incipient secondary growth. It has a uniseriate epidermis with a thick cuticle. The cortex is formed of 2 layers of collenchyma cells, middle 4-5 layers of chlorenchyma cells with starch grains, and inner parenchyma cells with several prismatic crystals and large sclereid bands. The cambial zone is indistinguishable, but evidently it gives rise to the outer phloem forming a narrow continuous strand and internal highly lignified secondary xylem. The vascular bundle is bi-collateral with intraxylary phloem. The pith consists of mucilage cells, starch grains and prismatic crystals (Figure 2).

T.S. of Mature Stem

T.S of mature stem shows a distinct cork layer comprising of 8-10 rows of uniformly arranged thick-walled cells (Figure 3). The cork cambium is distinct comprising of 4-5 layers of meristematic cells (Figure 4A). Secondary cortex is narrow and composed of 6-9 layers of thick-walled parenchymatous cells with several prismatic crystals (Figure 4 B-D). Beneath this layer is the broad vascular region. Growth ring boundaries are distinct in *Secamone emetica* stem. Secondary phloem intermingled with phloem fibers and phloem parenchyma is present above the vascular cambium (Figure 5). Xylem comprises of solitary vessels with helical thickening, vestured perforation plates, vasicentric tracheids and septate fibres. Presence of laticifers in

Figure 1: *Secamone emetica* (Retz.) R. Br. ex Sm. A. A vegetative branch exhibiting the aspects of the leaves. B. Fragment of stem bark.

rays is a characteristic feature of Apocynaceae. Medullary rays are uniseriate and heterocellular (Figure 6).

Powder Microscopical Study

The powder appears yellowish brown with no characteristic odour and taste. The powder shows fragments of cork cells in surface view, parenchyma cells with contents; bordered pitted vessels, fibres, fibrosclereids, and prismatic crystals (Figure 7).

DISCUSSION

The crucial things for consumers about drugs are purity, safety, potency and efficacy.[26] Macroscopic and microscopic observations are one of the best tools for formulating botanical identification of herbal drugs.[27]

The stems can exhibit both primary and secondary growth depending on the vegetal developmental stage.[28] The internal organization of the stem in the primary structure of *Secamone emetica* shows distinct uniseriate epidermis with thick cuticle, cortical region, vascular cylinder and medullary region. Similarly, Duarte and Larrosa (2011)[29] and El-fiki *et al.* (2019)[30] noticed

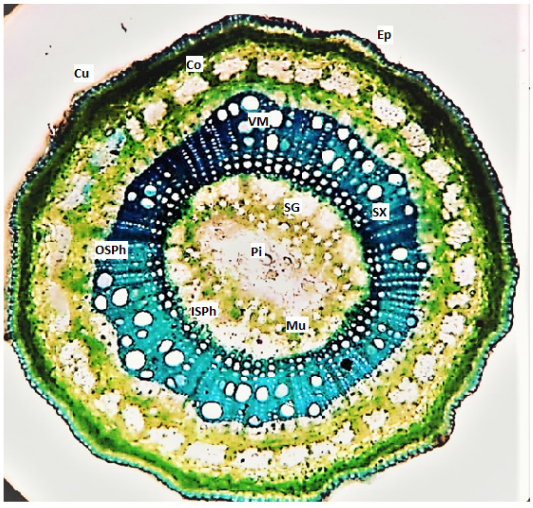

Figure 2: T.S of *Secamone emetica* young stem; Ep: Epidermis; Cu:Cuticle; Co: Cortex; OSPh: Outer Secondary Phloem; SX: Secondary Xylem; ISPh: Inner Secondary Phloem; VM: Vessel Multiples; Pi: Pith; Mu: Mucilage Cell; SG: Starch Grain.

thick cuticle in the stems of *Mandevilla coccinea* and *Carissa spinarum*, respectively. The vascular bundles in stems of the Apocynaceae family is usually bi-collateral; the xylem forms a continuous cylinder that is transversed by narrow rays, and the internal phloem always forms a continuous ring along the xylem or isolated bundles at the border of the pith.[31,32] These remarkable characteristics were also observed in *Secamone emetica.* Similar conclusions were drawn by Satos *et al.* (2010)[33] and El-fiki *et al.* (2019)[30] in stems of *Rauvolfia sellowii* and *Cryptostegia grandiflora*, respectively. The Apocynaceae family is also known for their small prismatic crystals and druses which are often distributed in the parenchymatic tissues.[31,32] These crystals are common in unlignified tissues[31] and were encountered in the cortical region of *Secamone emetica,* as observed in other species of family, such as *Plumeriopsis ahouai*,[34] *Forsteronia glabrescens*[35] and *Carissa spinarum.*[30] On the contrary, rosette and sandy crystals were noticed in *Beaumontia grandiflora* and *Alstonia scholaris*, respectively.[30]

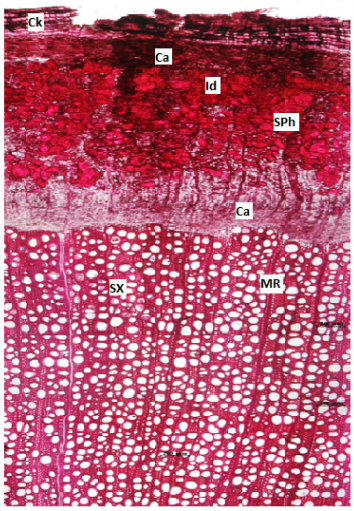

Figure 3: T.S of *Secamone emetica* mature stem; Ck- Cork, Ca -Cambium, Id-idioblast, SPh-Secondary phloem; SX-Secondary xylem, MR- Medullary Ray.

Presence of idioblasts containing phenolic compounds were observed in the cortical region of *Secamone emetica.* Likewise, Santos *et al.* (2010)[33] and Duarte and Larrosa (2011)[29] noticed idioblasts in stem of *Rauvolfia sellowii* and *Mandevilla coccinea*, respectively. Distinct growth rings in *Secamone emetica*

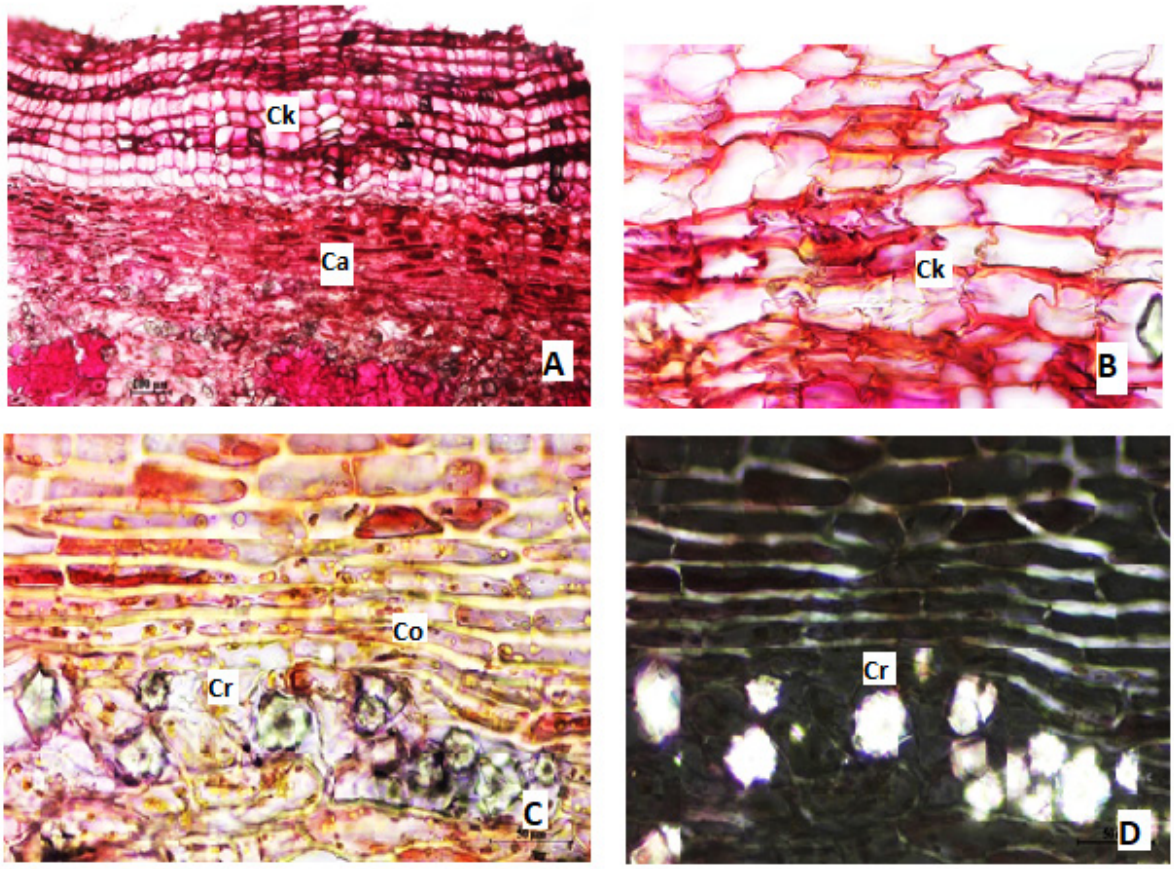

Figure 4: Enlarged portion of periderm and cortex; Ck-cork; Co-Cortex: Ca-cambium, CrCrystal.

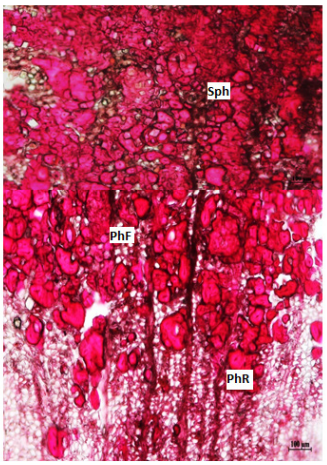

Figure 5: Enlarged portion of secondary phloem; PhR-Pholem Ray; Sph-Secondary Pholem; PhF-Pholem Fibre.

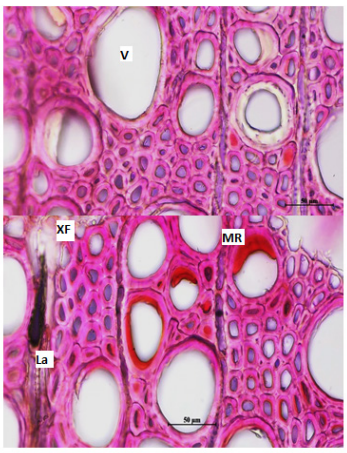

Figure 6: Enlarged portion of secondary xylem; V-Vessel; MR-Ray; XF-Xylem Fibre; La-Laticifers.

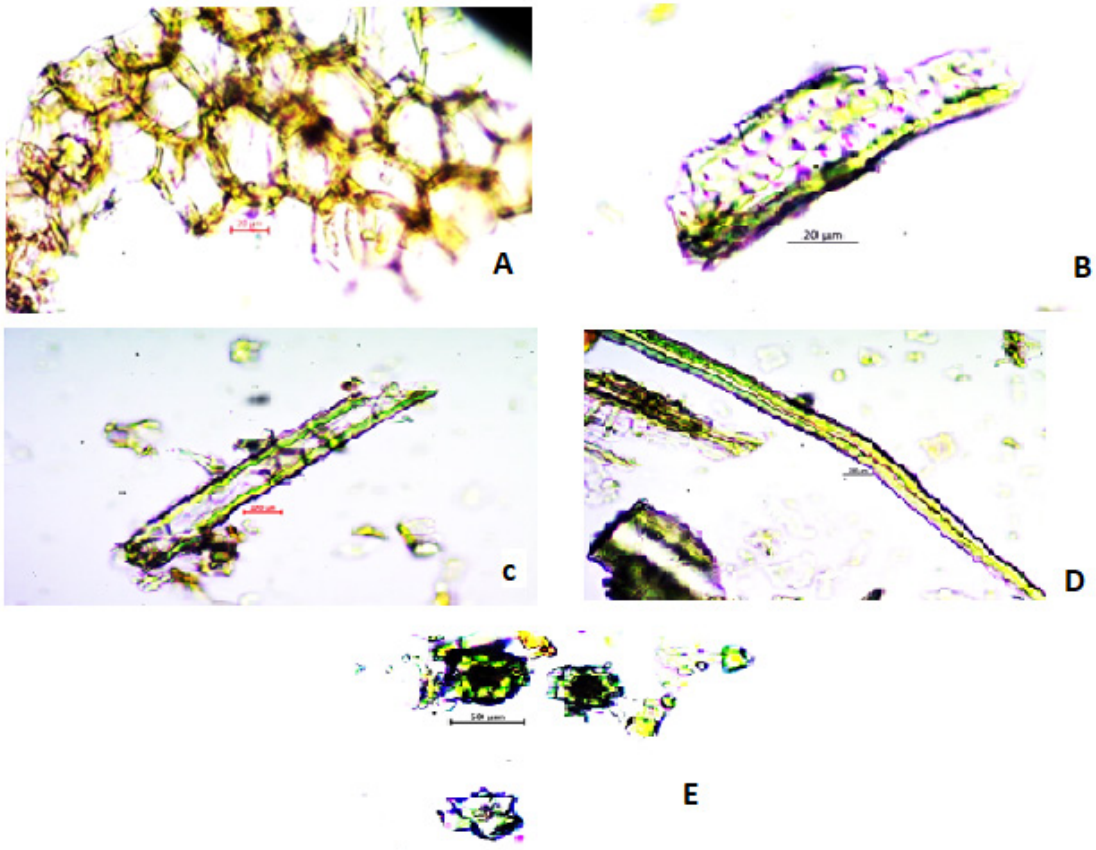

Figure 7: Powder microscopic studies of stem of *Secamone emetica*; A-Stem showing Cork cells; B-Bordered pitted vessel; C-Fragment of fibre; D-Fibrosclereid; E- Cluster crystals.

corroborates the results of Beckers *et al*[36] and Yaman and Tumen (2012).[37]

Metcalfe and Chalk (1950)[31] and Mauseth (1988)[38] stated that laticifers are common traits of the Apocynaceae family. Generally seen in the cortex, pericycle, phloem, pith, and occasionally in the medullary rays. The laticifers, typically larger than neighboring cells, display a prominent nucleus, and dense cytoplasm and in cross-section, exhibit a polygonal or circular shape without starch grains, akin to those verified in another species of the family.[35]

Secamone emetica showed the presence of laticifers with thick cell walls and dense cytoplasm, containing phenolic substances in medullary rays whereas Duarte and Larrosa (2011)[29] noticed laticifers in cortex, phloem and pith of *Mandevilla coccinea* and Santos *et al*., 2010[33] noticed laticifers in phloem and pith region of *Rauvolfia sellowii.*

Vestured perforation plates, vascicentric tracheids, and uniseriate heterocellular medullary were found to be indicative characters of Apocynaceae (Metcalfe and Chalk, 1950).[31] In *Secamone emetica* medullary rays are uniseriate and heterocellular. Similar to the present study uniseriate medullary rays were reported in *Marsdenia tomentosa* (Inside Wood, 2009).[39] On the contrary, Inside Wood (2009)[39] reported larger rays more than 10-seriate in *Leptadenia pyrotechnica*.

Powder microscopic studies of *Secamone emetica* showed the presence of some eragastic substances, cork cells, bordered pitted vessels, fragments of fibre, fibrosclereids and prismatic crystals. Akin to this, Adom *et al.* (2022)[40] noticed the presence of cork cells and prismatic calcium oxalate crystals in the powder of *Tabernaemontana crassa, Rauvolfia vomitoria* and *Voacanga africana*.

CONCLUSION

Little is known about wood anatomy of *Secamone emetica*. The described morpho-anatomical characters of *Secamone emetica* follow the Apocynaceae pattern and provide reliable data that can serve as a catalyst for future efforts to learn more about this endemic medicinal plant.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

%: Percentage; **ºC:** Degree Centigrade; **µm:** Milli micron; **cm:** Centimeter; **DPX:** Deparaffinated Xylene; **dia:** Diameter; **FAA:** Formalin acetic acid; **hr:** Hour; **mL:** Milliliter; **mm:** Millimeter.

SUMMARY

The research article explored the botanical characteristics of *Secamone emetica*, a climbing shrub from the Apocynaceae family, through macroscopic and microscopic analyses. The plant exhibited distinct features including its wiry and much-branched nature, milky-white latex and characteristic leaf, flower and fruit structures. Microscopically, the study revealed the primary and secondary stem structures, phenolic idioblasts, prismatic crystals and laticifers. Also, vestured perforation plates, vasicentric tracheids and uniseriate heterocellular medullary rays were noted as symptomatic features of Apocynaceae. The study accentuates the valuable insights for the precise identification of this plant and also aided in ensuring the purity, safety and efficacy of herbal medicines derived from *Secamone emetica*. The described morpho-anatomical characters of *Secamone emetica* follow the Apocynaceae pattern and provide reliable information that can serve as a catalyst for future efforts to learn more about this endemic medicinal plant.

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