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Research Article

Preparation and Evaluation of Oral Thin Films of a Natural Product: *Syzygium cumini* seed powder

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Abstract

Aim of this project is to develop oral thin films of *Syzygium cumini* seed powder. Though there were chewable tablets of *Syzygium cumini* reported in the literature, it has been chosen to formulate oral thin films of *Syzygium cumini* seed powder as they are expected to be more accepted by pediatrics, over the chewable tablets. Because of its high health benefits, these dosage forms have a potential to be nutraceuticals. Various phyto constituents present in the *Syzygium cumini* seed powder were screened through phytochemical screening. Four different formulations of oral thin films have been developed out of which two were optimized. Oral thin films were formulated with *Syzygium cumini* as the active ingredient and hydroxy propyl methyl cellulose, sodium alginate, glucose, guar gum, stevia, polyethylene glycol, water and dichloromethane were used as excipients. Different evaluation studies were performed for the oral thin films. Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed for the oral thin films to check the interactions between the active ingredients and the excipients. Antibacterial activity of the oral thin films was performed against two different bacterial species viz, *Escherichia coli* and *Bacillus subtilis.* It is concluded that the developed oral thin films were depicting antibacterial activity.

Keywords: *Syzygium cumini*, oral films, natural product, nutraceuticals, anti-bacterial activity

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INTRODUCTION

Syzygium cumini, often known as jambolan, Java plum, black plum, or jamun, is an evergreen tropical tree belonging to the Myrtaceae family of flowering plants[1,2]. Its natural range includes the Indian Subcontinent, Southeast Asia, China, and Queensland[3-5]. The fruit's name is occasionally mistranslated as blackberry, which is a distinct fruit in a different order. The fruit's seed is employed in Ayurveda, Unani, and Chinese medicine, among other alternative medical systems[6,7]. Table 1 lists the various phytochemical components found in Syzygium cumini seed powder. The fruit is also used to make wine and vinegar, and it contains a lot of vitamin A and C[8,9]. Other parts of this plant, such as the leaves, have been reported to have anti-platelet, anti-coagulant, anti-oxidant[10], anti-hypertriglyceridemic effect, anti-inflammatory properties, and anti-diabetic activity[11,12]. Syzygium cumini seed powder is reported to have nutraceutical properties as it contains significant amounts of carbohydrates, proteins, fats, fiber, calcium, and phosphorus[13-16]. Syzygium cumini seed powder contains phenols and tannins, which are primarily responsible for the seed powder's antibacterial properties. E. coli, Bacillus subtilis, Streptococcus, and Staphylococcus are among the bacteria that the seed powder is effective against[17-19].

Despite its enormous potential, there is only one formulation of Syzygium cumini seed powder have been reported to our knowledge[20]. This current study is an extension of our previously reported work, Palakurthi et al. It has been hypothesized that oral thin films can deliver actives rapidly through the sub lingual route by accelerating the absorption aided by the abundant blood vessel vasculature present below the tongue, which is an advantage over the oral chewable tablets. Moreover, oral thin films can also be administered by pediatric and geriatric populations as a nutraceutical, for whom chewing might be a limiting factor[20,21]. The active constituents of the seed powder depict anti-bacterial activity against various species of bacteria like *E. coli, Bacillus subtilis, Streptococcus* and *Staphylococcus, Pseudomonas, Lactobacillus and Atopobium species*, few of which also reside in the oral cavity. Four different oral thin film formulations were prepared by varied active ingredient and excipient composition. These preparations were characterized by performing quality control tests which include, weight variation, thickness, tensile strength, folding endurance, disintegration time and surface pH. Antibacterial activity was evaluated against *E. coli* and *Bacillus subtilis.*

Table 1: Phytochemical compounds identified in *Syzygium cumini* seed powder

S. No	Metabolite category	Compounds isolated
1	Flavonoids	Quercetin, rutin, 3,5,7,4-tetrahydroxy flavanone
2	Phenolic acids	Caffeic acid, ellagic acid, ferulic acid, gallic acid
3	Tannins	HHDP-galloyl glucose, trigalloyl glucose
4	Terpenes	Citronellol, geraniol, hotrienol, nerol, β-phenylethanol, phenylpropanal
5	Anthocyanins	Cyanidin, delfinidin, petudinin

MATERIALS AND METHODS

Materials

Seeds of *Syzygium cumini* were collected from domestically grown plant. They were shade dried, powdered and sieved. The authentication of the plant was obtained from Botanical Survey of India (BSI), Deccan Regional Center, Hyderabad, India. Hydroxy Propyl Methyl Cellulose (HPMC), sodium alginate, guar gum, polyethylene glycol (PEG) 400, glycerin, dichloromethane and ethanol were purchased from S.D Fine Chem Ltd. Mumbai, Maharashtra, India. Stevia powder was purchased from The Herbs N Spices, Neemuch, Madhya Pradesh, India.

Methods

Phytochemical screening: Phytochemicals like alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, cardiac glycosides and tannins were tested in the *Syzygium cumini* seed powder[20,22].

Formulation of oral thin films of *Syzygium cumini* seed powder: Oral thin films were formulated by solvent casting method. Briefly, Hydroxy Propyl Methyl Cellulose (HPMC) was dissolved in water, for F1, and sodium alginate was dissolved in water for F2 & F4, stirred until homogenous polymeric solution was formed. To the polymer solution, drug and Poly Ethylene Glycol (PEG) were added under continuous stirring. Other excipients such as glucose, guar gum (only in F3), stevia (only in F4) were added and allowed to stir for 30 min. The resulted solution was casted as films on glycerin coated petri-dish. The films were allowed to dry at room temperature for 4 hrs. Dried films were cut into desired size and shape as shown in fig 1. Solvent casting method for formulating oral thin films is schematically represented in fig 2. Table 2 explains the contents present in varied ratios in the formulated oral thin films.

Figure 1: Formulated oral thin films of *Syzygium cumini* seed powder

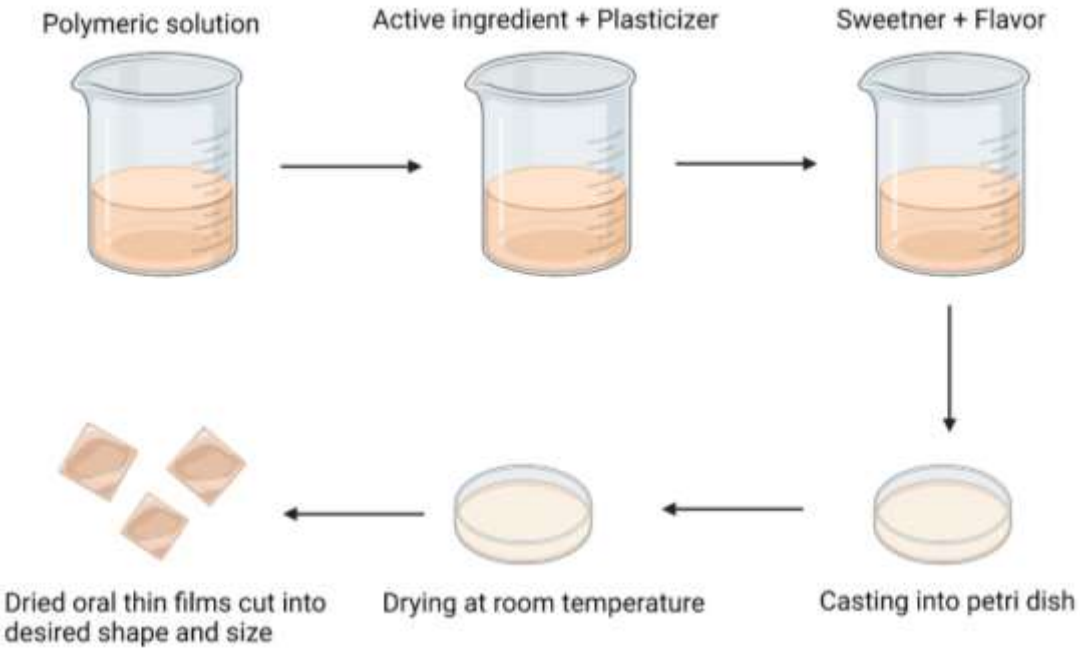

Figure 2: Schematic representation of formulating oral thin films of *Syzygium cumini* seed powder

Table 2: Formulations of oral thin films

Ingredients	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)
Seed powder	400	400	400	400
HPMC	450	-	-	-
Sodium alginate	-	150	-	150
Glucose	10	10	10	-
Guar gum	-	-	70	-
Stevia	-	-	-	10
PEG	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Water	-	Upto 10 ml	Upto 10 ml	Upto 10 ml
Dichloromethane	2 ml	-	-	-
Ethanol	Upto 10 ml	-	-	-

Evaluation tests for oral thin films:

Weight variation: 10 pieces of 2×1 cm film was cut from different locations and weight variation test was conducted. The average weight was calculated and compared to the weight of individual piece of the film.

Thickness: The thickness of the oral thin films of *Syzygium cumini* was measured by Vernier calipers and the average thickness was calculated.

Tensile strength: Tensile strength of the oral thin films was measured using a self-designed apparatus, which resembles the tensile strength apparatus. The apparatus was designed using a burette stand, two glass slides, weighing pan and some piece of thread. Tensile strength was calculated by the formula Tensile strength = Force at break (N)/ Cross sectional area (mm^2). Tensile strength is a measure of the mechanical strength of an oral thin film. Self-designed apparatus for measuring tensile strength was depicted in figure 3.

Figure 3: In-house custom designed tensile strength measuring apparatus

Folding endurance: Folding endurance is expressed as the number of folds required for breaking or developing visible cracks on the oral thin film. A strip of 2×1 cm film was subjected to this test by folding the film at the same point repeatedly several times until a visible crack was observed.

Disintegration time: This test was conducted by using disintegration test apparatus. A piece of film with an area of 2.25 cm^2 was placed in the basket, raised and lowered it in such a manner that the complete up and down movement at a rate equivalent to 30 times a minute. Time required for disintegration of the film where no traces of the film remain above the gauze were noted.

Surface pH: A piece of 2×1 cm film was placed in a petri-dish, to which 2 ml of water was added. The petri-dish was subjected to periodic shaking at a time interval of 20 seconds until the film was completely dispersed. pH of the resulting dispersion was measured using a digital pH meter. All the evaluation studies of optimized formulations (F2 and F4) were depicted in table 3.

Table 3: Evaluation of oral thin films

Parameter	F2 mean ± SD	F4 mean ± SD
Colour	Buff	Buff
Weight variation	Pass	Pass
Thickness	0.6 mm ± 0.03 mm	0.6 mm ± 0.03 mm
Tensile strength	0.45 ± 0.002 nm^2	0.45 ± 0.002 nm^2
Folding endurance	103 ± 4	105 ± 4
Disintegration time (min)	6.8 ± 0.3	7.1 ± 0.04
Surface pH	7.28 ± 0.04	7.30 ± 0.04

Study of antibacterial activity: Agar well diffusion techniques was acquired for calculating the zone of inhibition by the formulated oral thin films. Briefly, all the glassware used were sterilized in a hot air oven at 160˚C for one hour. The nutrient media was prepared and autoclaved at 120˚C for 20 min. Prepared media was allowed to solidify. Bacterial culture was inoculated by spread plate method and a well was made at the center using a sterile borer. Formulation was placed in the well, while the entire process was carried out in an aseptic chamber. These agar plates were incubated at 37˚C for 24 hrs. and the zone of inhibition was measured by a zone reader.

RESULTS AND DISCUSSION

Phytochemical screening: Standard procedures for detecting the phytochemicals present in the seed powder of *Syzygium cumini* were followed and the results were depicted in table. Phytochemicals such as alkaloid, flavonoids, tannins, cardiac glycosides, phenols and steroids/triterpenoids were tested to be present whereas saponins were found to be absent[20]. The results are depicted in table 4.

Table 4: Phytochemical screening of *Syzygium cumini* seed powder

S. No	Test	Result
1	Detection of flavonoids: 1) Shinoda Test	 Present
2	Detection of Cardiac glycosides: 1) Kedde's test 2) Baljet's test	 Present Present
3	Detection of Phenols	Present
4	Detection of saponins: 1) Froth Formation test	 Absent
5	Detection of alkaloids: 1) Mayer's Test 2) Dragendroff's test 3) Wagner's Test 4) Hager's Test	 Present Present Present Present
6	Detection of tannins	Present
7	Detection of steroids/ Triterpenoids: 1) Salkowski test 2) Sulphur powder test 3) Liebermann bucchard test	 Present Present Present

Phyto constituents like alkaloids, cardiac glycosides, flavonoids, steroids, tannins and phenols were found to be present whereas saponins were tested to be absent.

Preparation and evaluation of oral thin films:

Oral thin films of *Syzygium cumini* seed powder have been prepared by solvent casting method, depicted in figure. Composition of the oral thin films formulated is tabulated in table. Hydroxy Propyl Methyl Cellulose (HPMC) was used as a polymer in F1, where sodium alginate was used in F2 and F4. Formulation F3 does not have any polymer instead guar gum was incorporated. Glucose was used as a sweetening agent to mask the taste of the seed powder. In F4, stevia powder was used as a substituent for glucose as it does not have any impact on the blood sugar level, therefore diabetic patients could also use these oral thin films. When HPMC was used as a polymer in F1 thick films were formed. It was inferred not to use HPMC, sodium alginate was used as a polymer in further formulations. Four different oral thin films of *Syzygium cumini* formulations were made and evaluated for weight variation, thickness, tensile strength, folding endurance, disintegration time and surface pH.

All the evaluation tests have been performed for F2 and F4 as films were not formed with F1 and F4. The physical appearance of oral thin films was smooth and uniform with no cracks and the color was identified to be buff. Weight variation test was performed on the evenly cut pieces of oral thin films to ensure that the seed powder was uniformly distributed throughout an oral thin film. The weight variation of the oral thin films was found to lie between 0.11 to 0.15 g and these films pass the weight variation test as per USP. Thicknesses of the oral thin films were measured to be 0.6 mm ± 0.03 mm. The minimal standard deviation indicated that the method adapted for formulating the films was reproducible. Tensile strength of the oral thin films, measured using the self-designed instrument, was found to be 0.045 ± 0.002 nm^2. This value indicates plasticity of on oral thin film. Folding endurance was calculated to be 103 ± 4 for F2 and 105 ± 4 for F4. This is an indication that the thin films can withhold tension during packing and transportation.

Disintegration time should be short for oral thin films as the active ingredients needs to be released out from the formulation in the oral cavity itself. Disintegration time for F1 was 6.8 ± 0.3 min and that of F2 was 7.1 ± 0.04 min. Surface pH of F1 was measured to be 7.28 ± 0.04 and F2 was found to be 7.30 ± 0.04. The pH was intended to be neutral as acidic or alkaline pH cause oral irritation. Both F2 and F4 were found to be stable and meet the quality control standards for oral thin films of United States Pharmacopoeia (USP) as shown in table 4.

Interaction between the seed powder and the excipients of an oral thin film

To study the interactions between the active ingredient i.e. *Syzygium cumini* seed powder and the excipients present in the oral thin film formulation Fourier Transform Infrared Spectroscopy (FTIR) was performed (Fig 4 & Fig 5). FTIR spectra of pure *Syzygium cumini* seed powder and an oral thin film formulation are depicted in figure respectively. It can be inferred from the FTIR spectrum that there were no interactions between the active ingredient and the excipients of the oral thin film formulation as there was no major shift observed in the characteristic peaks of both the spectra.

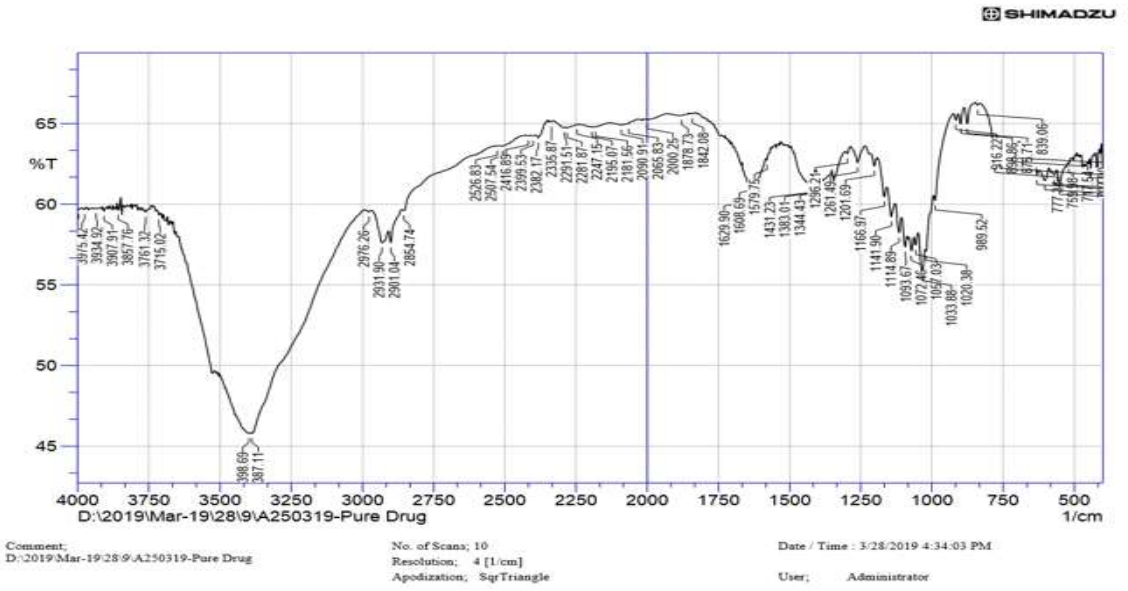

Figure 4: FTIR of *Syzygium cumini* seed powder

FTIR of the plain seed powder has been performed and it has been compared with that of the chewable tablet powder blend

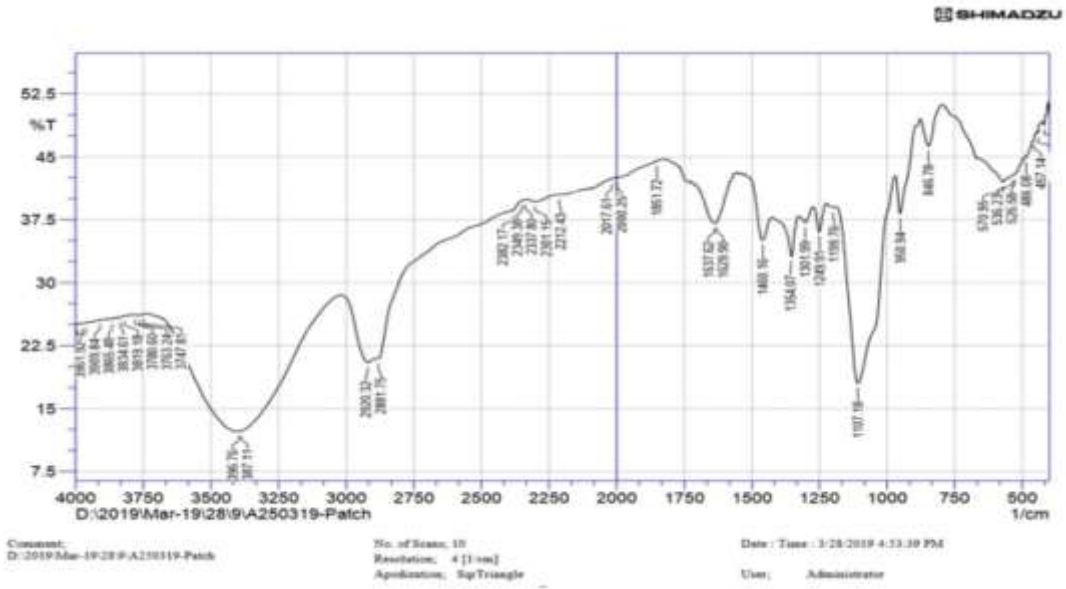

Figure 5: FTIR of oral thin film of *Syzygium cumini*

When the FTIR of the seed powder is compared to that of the oral thin film. It was observed there were no interactions between the seed powder of Syzygium cumini and the excipients of the oral thin film

In vitro antibacterial activity of an oral thin film against *Escherichia coli* and *Bacillus subtilis*

Formulated oral thin film and the active seed powder were studied for antibacterial activity against *E. coli* and *Bacillus subtilis* as these are the species of bacteria which reside mostly in the GIT and oral cavity. As the oral thin film starts to disintegrate in the oral cavity itself, there is a high possibility for an oral thin film to exhibit its bactericidal activity against the bacteria residing in the oral cavity. Both *E. coli* and *Bacillus subtilis* were actively inhibited our formulation. Zone of inhibition for *E. coli* was measured to be 3.37 cm and the zone of inhibition for *Bacillus subtilis* was recorded to be 2.49 cm. The results are depicted in fig 6 & fig 7 and tabulated in table 5.

Table 5: Antibacterial activity of oral thin films of *syzygium cumini* seed powder

S. No	Test component	Quantity (mg)	Zone of inhibition (cm)	
			Escherichia coli	*Bacillus subtilis*
1	Oral thin film	460	3.37	2.49
2	Seed powder	200	3.52	2.63

The anti-bacterial activity of both the seed powder and the formulated oral thin films have been tested against two different species of bacteria. Among the two bacteria, both oral thin films and seed powder showed more activity against *Escherichia coli* while compared to that of *Bacillus subtilis*

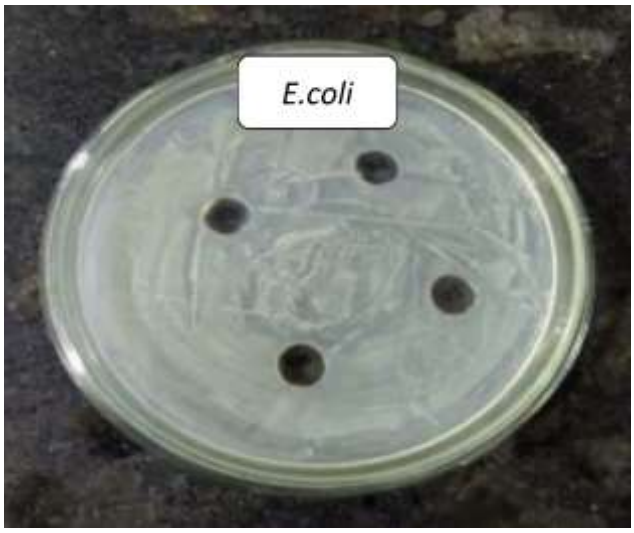

Figure 6: Anti-bacterial activity of *Syzygium cumini* oral thin film against *Escherichia coli*

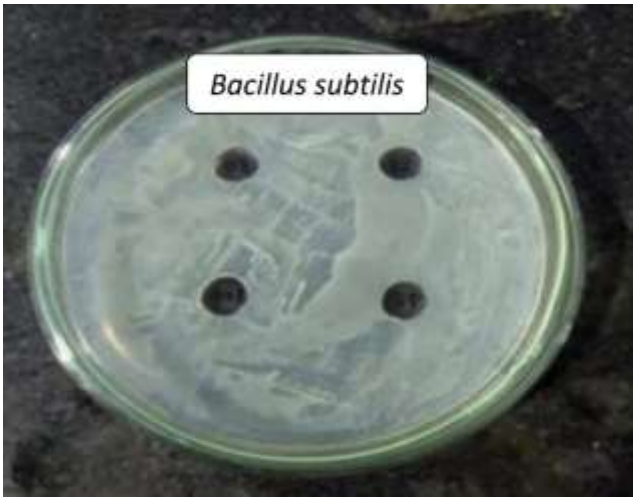

Figure 7: Anti-bacterial activity of *Syzygium cumini* oral thin film against *Bacillus subtilis*

CONCLUSION

Oral thin films of *Syzygium cumini* seed powder were formulated, evaluated and tested for their antibacterial activity. FTIR studies showed that there is no interaction between the active ingredient (seed powder) and the excipients. Antimicrobial test revealed that oral thin films were active against both gram-negative *Escherichia coli* and gram-positive *bacillus subtilis*. An oral thin film formulation with stevia powder can be administered by diabetic patients without affecting their blood sugar levels and these films can be potentially used as nutraceutical.

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Conflict of interest: The authors declare no conflict of interest.

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