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Formulation development and antimicrobial (in vivo) study of Eczema cream

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Abstract: *The increasing antimicrobial resistance disclosed by microorganisms causing superficial skin infections has led to extensive research on the therapeutic potential of Ayurvedic preparations. Medicinal plants contain many sorts of present and side effects-free anti-microbial compounds which will be effectively used against microbial infections. Eczema cream is one such polyherbal cream which contains Cassia tora (chakramard) , Azadirecta indica (neem), Pongamia pinnata (karanja) and seasume oil (til taila). As these drugs are already proven to have antimicrobial activity, considering this property an attempt has been made to prepare herbal cream with the help of modern techniques. The present study was intended at establish a standard profile of Eczema cream which was prepared using authenticated raw drugs with the help of modern techniques followed by detailed Pharmacognostical and physicochemical analysis asper standard protocol. By the evaluation it can be concluded that Eczema cream without side effects and having antimicrobial property can be used as provision of barrier to protect the skin and avoid various skin disorders like eczema, dermatophytosis, etc.*

Key Words: *anti microbial, Eczema cream, standard profile, chakramard, karanja, neem, etc.*

1. INTRODUCTION:

Superficial skin infections are very common in general practice and are caused by bacteria, fungi and viruses. Treatment of superficial skin infections is mainly based on the use of topical antibacterial and antifungal agents. However, the indiscriminate use of antimicrobial agents has led to antimicrobial resistance. The increasing antimicrobial resistance disclosed by microorganisms causing superficial skin infections has led to extensive research on the therapeutic potential of Ayurvedic preparations. Sneha Kalpana is a group of products of medicated taila and ghee, these drugs are treating very wide range of skin diseases among patients of all age groups. This formulation helps to transfer the aqueous and lipid-soluble active principles of all treated herbal drugs and material of animal and mineral origin. Chakramardadi oil is an proprietary medicine indicated in skin diseases like eczema, ringworm, dermatophytosis etc. The ingredients of chakramardadi oil are Cassia tora (Chakramard), Azadireacta indica (Neem) , Pongamia pinnata (Karanja) and Sesamum indicum(Til taila) (Table no.1). These ingredients are well known for their Kushtaghna, Kandughna, and Dadrughna effect. Chakramardadi oil is prepared by the same process as mentioned in ancient texts. As these drugs are already proven to have the antimicrobial effects(Table no.2), attempt has been made to develop the better drug for antimicrobial action as undesirable side effects of most of antibacterial & antifungal agents are important problems which needs to be solved. Therefore new & potent antimicrobial are essential to face the challenge pose by multidrug resistant micro-organism. So the present study aims to standardize the formulation of Eczema cream. As oil form of medicine posses certain inconvenience in case of its application, handling, packaging and transportation. The big hurdle while using oil form of medicine is it's sticky nature and while applied , it leaves stain over skin and clothes. So we should develope alternate form of drug which will be safe ,bioactive and potent. So oils can be converted into creams which will increase it's ease and shelf life. Cream is semisolid dosage form which need both oil and water in order to hydrate the skin efficiently to maintain the skin barrier composition. Cream provides better barrier to protect the skin than oil ,also helps in retention of moisture ,gives cleansing effect. The best advantage of cream dosage form of medicine over oil is it is less greasy, easy to wipe away and easy to apply. Hence to create the better and improved therapeutic application an attempt has been made to develop the cream form of chakramardadi oil for better acceptability and compliance.

2. MATERIALS AND METHODS-

Preparation of Chakramardadi oil:

Chakramardadi oil is prepared as mentioned in ancient text[1]. Seeds of Chakramarda, leaves of neem and seeds of karanja are taken cleaned, dried and a coarse powder was prepared. To prepare Kashaya 5 kg of Chakramarda seeds

(Cassia tora Linn) is made into coarse powder to which 40 liters of water is added and kept on heating device. Heating was continued on mandagni till it reduced to 1/4th. On filtration ten liters of Kashaya is obtained. This 10 liters kashaya with Chakramarda kalka, Neem kalka, Karanja kalka and Sesamum oil was subjected to sneha paka by heating on mandagni, till the taila paka siddha lakshanas were obtained. Then filtered in warm condition. Later taila was bottled and labelled.

Table 1: Composition of Chakramardadi oil

Sr no.	Ingredients	Botanical name	Form	Qty. Taken
1	Chakramarda	cassia tora	Kwath	1 kg
2	Neem	Azadirecta Indica	Kalka	250 gm
3	Karanj	Pongamia pinnata	Kalka	250 gm
4	Til tail	Sesame oil	Oil	4 lit

Images 1-Raw drugs of Chakramard oil

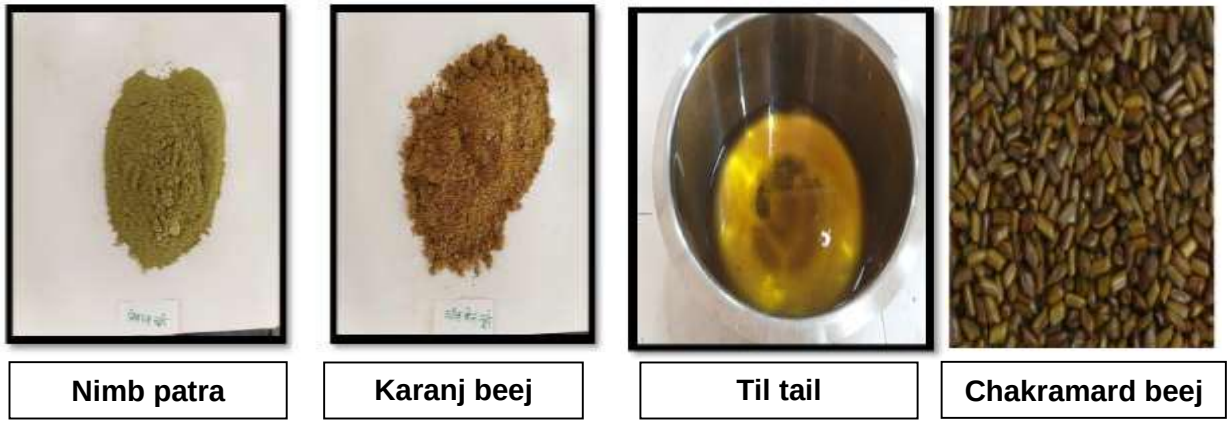


Table 2: Properties of ingredients[2,3,4]

Sr.no	Name of drug	Medicinal properties	Active compounds	Used in
1	Chakra mard	Guna (qualities) – Laghu–light to digest, Rooksha – Dryness Rasa (taste) – Katu –pungent Vipaka- Katu – Undergoes pungent taste conversion after digestion Veerya – Ushna – Hot potency Effect on Tridosha – Balances vata dosha	Fistucacidin, emodin, Rubro fusarin, Torosachrysone, Isotoralactone, Questin, Obtusin, Obtusifolin, Alaternins, Cassiaside etc. (JLN Shastry) Chrysophenol is its marker compound	Kushta – skin diseases Dadru – ring worm / tinea infection Pama – Keloids, Papules
2	Neem	Rasa – Taste – Bitter (tikta) and astringent (kashaya) Guna – qualities – Light to digest (Laghu), Dry (rooksha) Vipaka – Undergoes taste conversion into pungency after digestion. – Katu Vipaka Veerya – Potency – Cold in nature.	nimbin, nimbidin, nimbolide, limonoids Quercetin, ß-sitosterol	Kushta – skin diseases Dadru – ring worm / tinea infection Pama – Keloids, Papules
3	Karanja	Rasa (taste) – Tikta – Bitter, Katu (pungent), Kashaya (astringent) Vipaka – taste conversion after digestion – Katu (pungent) Veerya – Hot potency Guna (qualities) – Laghu (light to digest), Teekshna (piercing) Effect on Tridosha – Balances kapha and Vata.	Palmitic acid Stearic , Oleic acid, Linoleic acid, Linolenic acid , Arachidic acid, Eicosenoic acid, Behenic acid, Lignoceric	Kushta – skin diseases

4	Seasame oil	Rasa (taste) Madhura – sweet, Tikta – Bitter Anurasa – kashaya – Astringent sub-taste. Guna – qualities: Sukshma – minute, enters minute body channels Ushna – hot Vyavayi – gets absorbed and enters body channels very quickly. Teekshna – strong, piercing, Sara – eases bowel movements Vikasi – loosens joints Lekhana – scraping. Vipaka – Madhura – Undergoes sweet taste conversion after digestion Veerya – Ushna – Hot potency	Vitamin E Vitamin K. Sesame contains magnesium, copper, calcium, iron, zinc, and vitamin B6	Twachya – Good for skin Keshya – improves quality of hair.

Table 3: Quantitative analysis of ingredients[5,6]

Name of drug	Foreign matter mg %w/w	pH	Water soluble extractive %w/v	Alcohol soluble extractive %w/v
Chakramard	0.3%	5.89	30.2%	24.9%
Neem	0.2%	5.90	31.2%	25.6%
Karnaja	0.3%	5.92	30.1%	24.8%

Name of drug / Quantitative test	Sesame oil
Refractive Index	1.442
Specific Gravity	0.999
Acid value	1.7391
pH	6.12
Iodine value	106
Saponification value	190

Table 4: Analytical Values of Chakramardadi oil[7,8]

Sr. No.	Test	Result
1	Appearance	Clear oil
2	Colour	Greenish
3	Odour	Characteristic
4	Taste	Characteristic
5	Refractive Index	1.466
6	Specific Gravity	0.9078
7	Saponification value	191
8	Unsaponification Matter	1.9
9	Iodine Value	110
10	pH	5.8

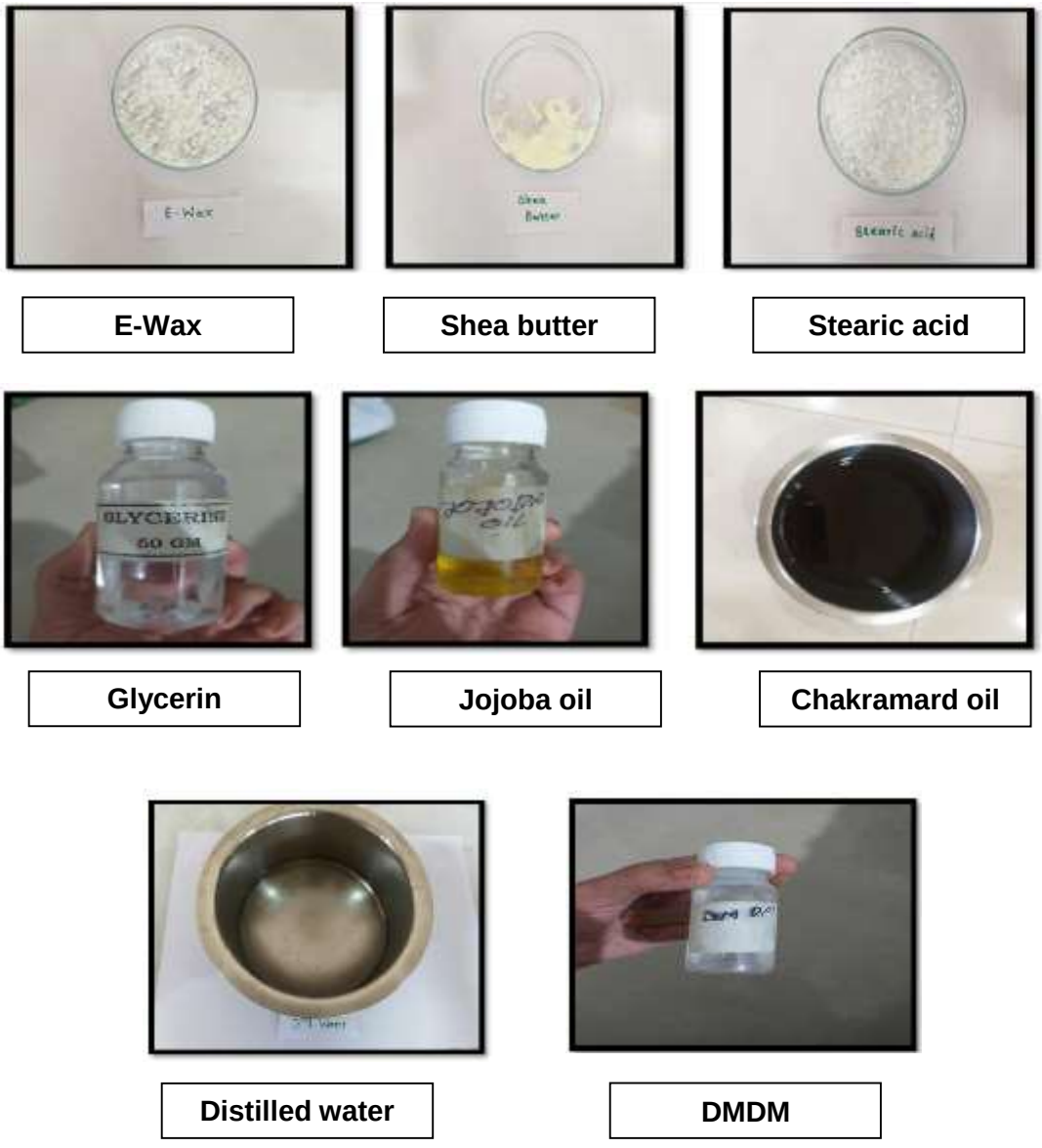
Preparation of Eczema cream:
Formulation synthesis is delineated in at table 5

Table 5: Composition of Eczema cream

SR NO.	Ingredients	Quantity in gm Batch F1	Quantity in gm Batch F2	Quantity in gm Batch F3	Quantity in gm Batch F4	Quantity in gm Batch F5
1	chakramardadi oil	130 ml.	130ml	130 ml	130 ml	130 ml
2	Water	600 ml	600ml	600 ml	600 ml	600 ml
3	Sheabutter	40 gm	40 gm	40 gm	40 gm	40 gm

4	E-wax	34gm	34 gm	34 gm	34 gm	34 gm
5	Steric acid	34 gm	34gm	34 gm	34 gm	34 gm
6	Glycerin	6gm	6gm	6 gm	6 gm	6 gm
7	Essence (Lavender oil)					
8	Methyl Paraben	1 gm	1 gm	1 gm	1 gm	1 gm
9	Propyl paraben	0.5 mg	0.5mg	0.5 mg	0.5 mg	0.5 mg
Total Quantity obtained		**675 gm**	**670gm**	**673 gm**	**672 gm**	**675 gm**

Images 2- Ingredients of eczema cream



2.1 EVALUATION OF CREAM

1. pH of the Cream
The pH meter was calibrated using standard buffer solution. About 5g of the cream was weighed and dissolved in 100 ml of distilled water and its pH was measured.
2. Viscosity
Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no 7.
3.Homogeneity
The formulations were tested for the homogeneity by visual appearance and by touch.
4.Appearance
The appearance of the cream was judged by its color, pearlscence and roughness and graded.

5.After feel

Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

6.Type of smear

After application of cream, the type of film or smear formed on the skin were checked.

7.Removal

The ease of removal of the cream applied was examined by washing the applied part with tap water.

8.Acid value

Take 10 gm of substance dissolved in accurately weighed, in 50 ml mixture of equal volume of alcohol and solvent ether, the flask was connected to reflux condenser and slowly heated, until sample was dissolved completely, to this 1 ml of phenolphthalein added and titrated with 0.1N NaOH, until faintly pink color appears after shaking for 30 seconds.

Acid value = n*5.61/w

n - number of ml of NaOH required, w - weigh of substance.

9.Saponification value

Introduce about 2 gm of substance refluxed with 25 ml of 0.5 N alcoholic KOH for 30 minutes, to this 1 ml of phenolphthalein added and titrated immediately, with 0.5 N HCL.

Saponification value = (b-a)*28.05/w

a - volume in ml of titrant, b - volume in ml of titrant, w - weigh of substance in gm

10.Irritancy test

Mark an area (1sq.cm) on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24 hrs and reported.

11. Accelerated stability testing

Accelerated stability testing of prepared formulations was conducted for 2 most stable formulations at room temperature, studied for 7 days. They were formulations no 2 and 4 at 40 oC ± 2 oC for 20 days. The formulations were kept both at room and elevated temperature and observed on 0th, 5th, 10th, 15th and 20th day for the following parameters.

3. RESULTS:

pH of the Cream

The pH of the cream base was found to be in range of 6.2-6.8 which is good for skin pH. All the formulations of cream base were shown pH nearer to skin required (Table 6).

Table 6: Determination of pH of prepared cream

Formulations	PH
F1	6.4
F2	6.2
F3	6.6
F4	6.8
F5	6.7

Viscosity

The viscosity of was cream was in the range of 27023-27051 cps which indicates spreadibilty of cream. In our study F1, F2, F3 ,F4, F5 depicted easily spreadable property.

Acid value and saponification value

The results of acid value and saponification value of all formulation of cream base were presented in table 7, and showed satisfactorily values.

Table no 7: acid value and saponification value

Batch No	F1	F2	F3	F4	F5
Acid value	5.8	5.9	6.2	5.7	6.1
Saponification value	25.5	25.9	24.9	25.2	25.6

Irritancy test
The formulation shows no redness, edema, Inflammation and irritation during irritancy studies. These formulations are safe to use for skin (Table 8).

Table 8: Type of adverse effects of cream

Formulations	Irritant	Erythema	Eczema
F1	Nil	Nil	Nil
F2	Nil	Nil	Nil
F3	Nil	Nil	Nil
F4	Nil	Nil	Nil
F5	Nil	Nil	Nil

Homogeneity
All formulations of base produce uniform distribution in cream. This was confirmed by visual appearance and by touch (Table 9).

Appearance
When formulation were kept for long time, it found that no change in colour of cream base (Table 9).

After feel
Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream base was found (Table 9).

Type of smear
After application of cream base, the type of smear formed on the skin were non greasy (Table 9).

Removal
The cream applied on skin was easily removed by washing with tap water (Table 9).

Table 9: Organoleptic characters of Eczema cream

Parameters	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Color	Light creamish	Light creamish	Light creamish	Light creamish	Light creamish
Odor	Aromatic	Aromatic	Aromatic	Aromatic	Aromatic
Touch	Greasy	Greasy	Greasy	Greasy	Greasy
Consistency	Smooth, soft	Smooth, soft	Smooth, soft	Smooth, soft	Smooth, soft
Texture	Smooth, Creamy, Thick, Non coarse	Smooth, Creamy, Thick, Non coarse	Smooth, Creamy, Thick, Non coarse	Smooth, Creamy, Thick, Non coarse	Smooth, Creamy, Thick, Non coarse

Table 10: Analytical Values of Eczema cream

Sr. No.	TEST	RESULT
1	Appearance	Semi Solid Soft Cream
2	Colour	White
3	Odour	Characteristic
4	Taste	Slightly Bitter

5	LOD	10.85
6	pH	6.4
7	Melting Point	49
8	Spreadability	Uniformly Spreadable
9	Uniformity of content	Complies
10	Density	0.7548

3.1 ANTIMICROBIAL STUDY OF ECZEMA CREAM:

The Agar well diffusion method is used to evaluate the antimicrobial activity of the prepared herbal cream. In the present study Muller Hinton(MH) is chosen as culture media for the bacteria and potato dextrose (PD) agar media for fungal. Candida albican, Aspergillus Niger, Staphylococus aureus, Pseudomonas auregenosa, Acinobacter baumanii were used for antimicrobial activity. Flacunazole and amoxicillin used as standard drug for fungal and bacterial study respectively. Growth of organisms confirmed by the turbidity of media.

Loopful of the microorganisms are emulsified in 100 ml sterile growth media under proper sterile condition and incubated for 72 hrs at 37℃ in incubator. This prepared inoculums are added to 45 ml of flask containing MH agar and PD agar at 37℃. This is immediately poured into dry sterile petridish to a depth of 8mm. The Petri dishes are placed to leveled surface to ensure that the layers of medium are of uniform thickness. Allow the plates to solidify at the room temperature for 12 hrs. Incubated some plates at 350℃ to check the sterilty.

Table no 11 : Prepration of culture media (Muller Hinton Agar)

Ingredients	In gm /liter
Beef extract	2.00 gm
Acid Hydrolysate of casein	17.50 gm
Starch	1.50 gm
Agar	17.00 gm
Distilled water	1000 ml

Table no 12: Preparation of culture media (potato dextrose agar)

Ingredients	In gm /liter
Potato starch	4.00 gm
Dextrose	20.00 gm
Agar	15.00 gm
Distilled water	1000 ml

The zone of inhibition of bacterial growth around the cup/well measured in mm with the help of scale. The readings were taken at four different planes and then mean was calculated. The results are shown in Table 13 and table 14.

Table no 13 : Anti fungal study -Zone of inhibition in mm

Sr no.	Test organism	Zone of inhibitions (mm)		
		Control drug DMSO	Standard drug Fluconazole (150 ug/ml)	Eczema cream
1	Candida albican	No zone of inhibition	No zone of inhibition	13 mm
2	Aspergillus Niger	No zone of inhibition	16mm	12mm

Table no 14 : Anti-bacterial study -Zone of inhibition in mm

Sr no.	Test organism	Zone of inhibitions (mm)		
		Control drug DMSO	Standard drug Amoxicilin (250 mg/ml)	Eczema cream
1	Staphylococus aureus	No zone of inhibition	45mm	12 mm

2	Pseudomonas aeruginosa	No zone of inhibition	No zone of inhibition	41 mm
3	Acinobacter baumanii	No zone of inhibition	No zone of inhibition	No zone of inhibition

Images 3- antimicrobial activity

Staphylococcus aureus

Staphylococcus aureus

Pseudomonas auregenosa

Pseudomonas auregenosa

Acinetobacter baumannii

Candida albicans

4. DISCUSSION :

Eczema cream is properitory medicine which is used in different skin disease. This herbal cream contains Azadirecta indica, Cassia tora, Pongamia pinnata and seasame oil as active ingredientes. Purpose of the study is to formulate the herbal cream which shows similar antimicrobial property as of modern medicine and is side effects free. As these drugs are already proven to have antimicrobial properties, and seasame oil is also useful in skin diseases , taking all these points into consideration choice of ingredientes is made . Preparation of cream is done in 5 batches followed by their physicochemical analysis. By the results it is made clear that the formulation has light creamish colour with smooth and soft consistancy . The formulated herbal cream has viscocity in the range of 27021 to 27053 which makes it easily spreadable. By the application of this herbal cream it forms nongreasy layer over the skin making it easily removeable with tap water.The pH of prepared cream was nearer skin pH, and cream produces homogeneous, emollient, non-greasy and easily removed properties after the application. The herbal cream was safe in respect to skin irritation and allergic sensitization. The prepared herbal cream was also evaluated for its antimicrobial activity against various strains of microorganisms. Results shows that Eczema cream posseses antibacterial activity againt Staphylococus aureus and Pseudomonas aeruginosa indicating inhibition zone of 12mm and 41mm respectively when compared with the standard drug Amoxicilin at the conc.of 250 mg/ml. The cream doesn't show any zone of inhibition against bacteria Acinobacter baumanii. Similary the prepared herbal cream evaluated for its antifungal activity agianst Candida albicans and Aspergillus Niger showing inhibition zone of 13mm and 12 mm respectively. For the antifungal activity flacunozole is used as standard drug at the conc.of 150ug/ml. The eczema cream gave zone of inhibition >10mm showing strong antimicrobial activity. The prepared herbal antimicrobial cream is intended for medicinal use rather than as other cosmetic. These studies suggest that herbal antimicrobial cream is more stable and also it may produce synergistic action.

5. CONCLUSION:

Eczema cream is polyherbal medicated herbal cream used externally for treating eczema, dermatophytosis and other skin infections. The findings of present investigation exhibited that the prepared herbal cream containing extracts of Cassia tora, Azadirecta indica and Pongamia pinnata were safe to use in skin. By the analytical testing study revealed the standard profile of Eczema cream and In Vivo study proves the antimicrobial activity of prepared herbal cream

Further to determine whether the preparation can be effectively used in superficial skin infections, a case control study on humans should be done.

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