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THE CONCEPT OF ANTIMICROBIAL ACTIVITY IN *AYURVEDA* AND EFFECT OF ARKADI GANA ON GRAM NEGATIVE BACTERIA

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ABSTRACT

Purpose: To evaluate the concept of antimicrobial activity & gram-negative activity of the *Arkadi gana.* **Introduction:** In *Ayurveda* diseases are broadly classified into exogenous and endogenous based on etiological origin. Micro-organism exercises a major part among the exogenous categories. Microorganisms in the external environment have existed on earth for 3.5 billion years.It is quite natural that *Ayurveda*, the oldest health care system in the world, will not have the word 'Antibiotics'. Various types of diseases produce by these organisms and modes of spread are also described in *Ayurveda* like *Kustha, Jvara, Sosa (*consumption*), Netrabhisyanda (*conjunctivitis*)* and *Opsargic roga (*Infectious disease*).*The treatment of these disease are *Rakshoghana Karma*, *Manidharna*, *Dhupan* etc are prescribed in *Ayurveda*. **Methods**: For antimicrobial activity aqueous, ethanol (Rotatory shaker method) and petroleum ether extracts (Soxhlet Apparatus) were prepared as per API guidelines and tested on different species of common pathogenic bacteria that's responsible for wound infection. Anti microbial study was done by 12 samples against 4 bacteria. *In vitro* studies were undertaken to assess the antibacterial activity at Institute of biomedical and Industrial research, jaipur. **RESULTS:** Disc diffusion method was employed and zone of inhibition are measured. Results are analyzed statistically, with one way anova and dunnett's multiple comparisons test are applied. **Discussion & Conclusion:** *Arkadi gana* are strong antibacterial agent against all bacteria, these pathogens are responsible for wound infections, osteomyelitis and associated with

dysentery, diarrhoea, gastroenteritis, urinary tract infection and skin infections (impetigo, folliculitis).

KEYWORDS: *Arkadi* gana, antimicrobial activity, *Ayurveda*.

INTRODUCTION

Sushruta Samhita is the basic text book of *Shalya Tantra* (Surgery) in which *Acharya Sushruta* mentioned the importance of *Vrana* in different context as in the definition of *Shalya Tantr.*[1] (*Vrana Vinischayarth Su.Su. 1/9*), while describing the importance of *Vaidy.*[2] (*Su.Su.17/11*) and in *Shashthi Upkarma* of *Vran.*[3] *(Su. Chi1/8).*

Many a times, non-healing *Vrana* pose a problem in surgical practice. Healing of *Vrana* is a natural process, but due to the interference of vitiated *Doshas*, *Vrana* becomes *Dushta* and normal healing process gets delayed.[4]

In modern science wound is the commonest painful condition that every human being suffers in their life and skin is the largest organ in body. It is the chief site of secondary infection being main portal entry for bacteria, viruses etc. So it is the responsibility of surgeon that it should be cured within short period with less cost and less pain.

Surgical infection, particularly surgical site infection (SSI), has always been a major complication of surgery and trauma and has been documented for 4000–5000 years. Many references suggested that microbes are responsible for contamination and delayed wound healing, and uses of anti-microbial agents are very important for prevention of sepsis. Like the Hippocratic teachings described the use of anti-microbial, such as wine and vinegar, which were widely used to irrigate open, infected wounds before delayed primary or secondary wound closure, medical papyruses also described the use of salves and antiseptics to prevent surgical sight infection.[5] Amount of tissue injury and degree of contamination influences the speed and quality of healing.

In terms of *Nidana*, external factors or *Nimitta Karana* is to be implied here and the qualitative knowledge of compatible and incompatible environment, diet and regime are very much important. The classified *Nijaroga* and *Aguntuja roga* are caused due to *Samanya Karana* like *Mithya Ahara-Vihara* and *Visista Karana* like poison, weapon, worm, insects, wild animal etc.[6] In the ancient period the people were not susceptible to the micro-organism. But, with the advent of time, the prevalence of common diseases were observed

due to the influence of specific micro organisms and thus the concept of micro-organism became an established phenomenon and is regarded as the external factors for the production of diseases.

The understanding of the causes of infection came in the nineteenth century. Microbes had been seen under the microscope. The concept of a 'magic bullet' (*Zauberkugel*) that could kill microbes but not their host became a reality with the discovery of sulphonamide chemotherapy in the mid-twentieth century. After the discovery of the antibiotic penicillin the infection has been controlled significantly. The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases.

NEED OF STUDY

Experimentation is the stepping stone for the advancement. It is based on trial and error method. To study the therapeutic effects of drugs, experimentations are carried out. As experimentations on human beings are not ethical or possible, therefore preliminary experiments are to be conducted to evaluate the efficacy followed modern parameters and scientific tools, toxicity and after also be conducted on the animals.

Antimicrobial agents are among the most commonly used and misused in all drugs, Resistance developed and toxic effects to the human beings. The attention is now being diverted for searching alternate drugs for antimicrobial property (*Krimihara* and *Vrana Shodhana*) to find new solutions for the infectious disease like wound infection and at some time does not produce toxic effect.

AIMS AND OBJECTIVE

- To evaluate Concept of antimicrobial in *Ayurveda* in concordance with modern medicine.
- To standardize and evaluate the efficacy of *Arkadi gana* extracts on Gram negative bacteria.
- A comparative anti microbial activity against potent antibiotics.

Antimicrobial activity in *Ayurveda*

The concept of micro organism as causative factor for the production of disease where classified in *Samhitas* as *Krimi* .The word *'Krimi'* has very much potential in *Ayurvedic* literature & reflect them in vast dimension. Visible or invisible minute animals that affect on

living & non living things of biosphere are described very efficiently in *Ayurvedic* science. It includes all types of macro and micro pathogenic and non pathogenic organisms.

Apakarsana, *Prakrtivighata* and *Nidana-parivarjana* are the principles of treatment mentioned in context to combat the *Krimi* (parasitic infections). But the same are also applicable for the cure of all the diseases caused by microbial infections. Therapeutically *Sodhana*, *Samana* and *Nidana-parivarjana* are the respective terminologies implied in context to anti-microbial activity.[6] Siro*virecana*, *Vamana*, *Virecana* and *Asthapana* are applicable for *Apakarsana*[7]

Administrations of *Arkadi Gana* are used for the said respective therapies. Simultaneously antagonist drug therapy for destruction (cidal) or limitation of the cause (static) is applied for *Prakrtivighata.* The anti-microbial activity incorporates *Visaghna*,*Vranasodhana*, *Vranaropana* and *Kled-puyopasosana* activities. The ultimate aim is to arrest and encounter the infection. For these to encounter the caused due to specific micro-organism is to be identified and accordingly the stipulated drug from *Krimighna* and or *Visaghna.*

EXPERIMENTAL STUDY

1. MATERIALS AND METHODS

1. अर्कादि गण

अर्कालर्ककरंजद्वयनागदन्तीमयूरकभारंगीरास्नेन्द्रपुष्पीक्षुद्रश्वेतामहाश्वेतावृचिकाल्यलवणास्तापसवृक्षश्चेति
अर्कादि गणो ह्येष कफमेदोविषापहः ।

कृमिकुष्ठप्रशमनो विशेषाद् व्रणशोधनः ।। सु० सू० **38/16-17**

The *Arkadigana* alleviates *Kapha, Meda, Visa, Krimi* And *Kustha*. It is specially purifying or cleaning wounds and ulcers.

Table 1.List of Plants, Botanical Name and Useful Parts.

Sr.No.	PLANT NAME	BOTANICAL NAME	FAMILY	USEFUL PARTS
1	*ARK –RAKTA*	*Calotropis gigantia*	*Asclepiadaceae*	Mool
2	*ARK –SHVETA*	*Calotropis procera*	*Asclepiadaceae*	Mool
3	*KARANJA*	*Pongamia pinnata*	*Leguminosae*	Twak
4	*KANTKI KARANJA*	*Caesalpinia crista*	*Leguminosae*	Beej
5	*NAGDANTI*	*Croton oblongifolia*	*Euphorbiaceae*	Mool
6	*MAYURAKA*	*Achyranthes aspera*	*Amaranthaceae*	Mool
7	*BHARANGI*	*Clerodendrum serratum*	*Verbenaceae*	Mool
8	*RASNA*	*Pluchea lanceolata*	*Compositeae*	Patra
9	*INDRAPUSHPI*	*Gloriosa superba*	*Liliaceae*	Mool

10	*KSHUDRA SHWETA**			
11	*MAHA SHWETA***			
12	*VRISCHIKALI*	*Pergularia extensa*	*Asclepiadeceae*	Whole plant
13	*ALAVANA*	*Celastrus panniculatus*	*Celastraceae*	Beej
14	*TAPASVIKSHA*	*Balanites aegyptiaca*	*Simaroubaceae*	Twak

Note - * & ** plants are Now unavailable and some controversy are the identification and nomenclature. 12 drugs are select for present study.

2. Identification and Authentication of drugs

Identification of plants species were authenticated by referring standard literature and PG department of *Dravya Guna* NIA Jiapur. Authentications of raw drugs were done by department of botany Rajasthan University Jaipur.

3. Powder Formation

Collected plant parts were shade dried and ground to a fine powder using grinder mixer.

(2) Selection and Collection of Pathogens

(A) *Proteus mirabilis.*[8]

Proteus is a rod-shaped, gram-negative bacterium that inhabits the intestinal tracts of humans and animals. It can be found in soil, water and fecal matter. Known to cause urinary tract infections and wound infections.

(B) *Escherichia coli*

E. coli is gram-negative, facultative anaerobic and non-sporulating. It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. The most common infection is urinary tract infection, diarrhoea or gastroenteritis, pyogenic infections and septicemia.

(C) *Klebsiella aerogenes*

Klebsiella is a gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines. The most common infection caused by *Klebsiella* bacteria outside the hospital is pneumonia.

(D) *Pseudomonas aeruginosa*[9]

Pseudomonas aeruginosa is a gram-negative, aerobic, rod-shaped bacterium with unipolar motility. It is found in soil, water, skin flora and most man-made environments throughout

the world. *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections.

(3) Preparation of the extracts[9]

1) Aqueous and ethanol extract

5 g of the air dried drug, ade100 ml of distil water in a closed flask for twenty-four hours, kept on a rotary shaker at 190-220 rpm shaking frequently during six hours and allowing standing for eighteen hours. Filter rapidly, taking precautions against loss of solvent and dry at 100ºC, to constant weight and weigh. And dissolve 15 ml distil water and collected air tight moisture free zip seal plastic cover.

2) Preparation of petroleum extract

Use continuous extraction methods (Soxhlet Apparatus). The powdered 10gm sample was placed in a thimble and plugged with cotton wool. The flask containing 100 ml solvents was attached Below with Soxhlet apparatus at a time and condenser above. In this apparatus, extraction is by boiling solvent followed by percolation. So, it was carried out at the boiling point of solvent i.e. at 60° C. Solvent was gradually refluxed into thimble and siphoned into the flask. In this way, it has taken minimum 5 cycles for complete exhaustion of the samples. After complete exhaustion, the material was removed, the assembly readjusted and maximum solvent was recovered. Then transferred in a glass Petri dish and evaporated to dryness at 40°C and kept in a moisture free zip seal plastic cover.

(2) ANTIBACTERIAL ACTIVITY

1. Media plate Preparation for growth of bacteria
    1. Muller Hilton agar- For *E.coli* & *Pseudomonas aeruginosa*.
    2. Blood agar - For *Klebsilla aerogenes*.
    3. Corn meal agar- For *Proteus mirabilis*.

2. Incubation[11]

Stored in the BOD incubator, 37ºC to complete growth of bacteria. After complete growing used for disk diffusion susceptibility testing.[12]

Place the disks individually with sterile forceps or with a mechanical dispensing apparatus, and the gently press down onto the plate.

3. Measured the diameter of the zones of complete inhibition

The zones of growth inhibition compared and recorded as susceptible, intermediate, or resistance to each drug tested. Colonies growths within the clear zone of inhibition may represent resistant variants or mixed inoculums. Water, ethanol and petroleum ether are used for negative control activity against test organism. Vancomycin.[13] (7.5mg w/v) per disk serves as a positive control. Sample (*Arkadi gana* as compound 7.5mg w/v) aqueous, ethanol and petroleum ether extract were used.

OBSERVATION AND RESULTS

TABLE NO: 2. ANTIMICROBIAL ACTIVITY (ZONE OF INHIBITION) OF POSITIVE CONTROL, NEGATIVE CONTROL AND DIFFERENT EXTRACTS OF "*ARKADIGANA*" AGAINST DIFFERENT MICROORGANISM

Sr. No	Micro-organism	Positive Control Zone of Inhibition (mm)	Negative control (Water, Ethanol& Pet. ether)	Sample (Extracts)	Sample Zone of inhibition(mm)			
Antibacterial sensitivity Vancomycin [7.5mg(w/v)]					**1st**	**2nd**	**3rd**	**Mean**
1.	***Proteus Mirabilis***	27mm	0 mm	Aqueous	10mm	11mm	10mm	10.33 mm
2.		27mm	12 mm	Ethanol	15mm	16mm	15mm	15.33 mm
3.		27mm	13 mm	Pt. Ether	17mm	17mm	16.5mm	16.83 mm
4.	***E. coli***	25mm	0 mm	Aqueous	11mm	11mm	11mm	11 mm
5.		25mm	12 mm	Ethanol	16mm	17mm	19mm	17.33 mm
6.		25mm	13 mm	Pt. Ether	19mm	19mm	18.5mm	18.83 mm
7.	***Pseudomonas aeruginosa***	25mm	0 mm	Aqueous	10mm	11mm	10mm	10.33 mm
8.		25mm	12 mm	Ethanol	12mm	13mm	14mm	13 mm
9.		25mm	13 mm	Pt. Ether	16mm	16mm	16mm	16 mm
10.	***Klebsilla aerogenes***	17mm	0 mm	Aqueous	10	11	10	10.33 mm
11.		17mm	0 mm	Ethanol	11mm	12mm	11mm	11.33 mm
12.		17mm	0 mm	Pt. Ether	11mm	14mm	13mm	12.66 mm

Table No. 3. Showing Antimicrobial Activity of '*Arkadigana*' Against *Proteus mirabilis*

Proteus mirabilis	**NEGATIVE mean ± s.e.m.**	**SAMPLE mean ± s.e.m.**	**POSITIVE mean ± s.e.m.**	**P. VALUE**	**SIGNIFICANCE**
Aqueous	0±0	10.33±0.0577	27±0		
Ethanol	12±0	15.33±0.0577	27±0	<0.0001	H.S.
Pt. Ether	13±0	16.83±0.02887	27±0	<0.0001	H.S.

Dunnett's multiple comparisons test (ET)	**Mean Diff.**	**95% CI of diff.**	**Significant?**	**Summary**
Negative vs. sample	-3.333	-4.113 to -2.554	Yes	****
Negative vs. Positive	-15.00	-15.78 to -14.22	Yes	****

Dunnett's multiple comparisons test (PE.)	Mean Diff.	95% CI of diff.	Significant?	Summary
Negative vs. Sample	-3.333	-4.113 to -2.554	Yes	****
Negative vs. Positive	-15.00	-15.78 to -14.22	Yes	****

Table No. 4. Showing Antimicrobial Activity of '*Arkadigana*' Against *E. coli*

E. coli	NEGATIVE mean ± s.e.m.	SAMPLE mean ± s.e.m.	POSITIVE mean ± s.e.m.	P. VALUE mean ± s.e.m.	SIGNIFICANCE
Aqueous	0±0	11±0.0577	25±0		
Ethanol	12±0	17.33±0.0346	25±0	<0.0001	H.S.
Pt. Ether	13±0	18.83±0.0288	25±0	<0.0001	H.S.

Dunnett's multiple comparisons test (ET)	Mean Diff.	95% CI of diff.	Significant?	Summary
Negative vs. Sample	-4.200	-4.668 to -3.732	Yes	****
Negative vs. Positive	-13.00	-13.47 to -12.53	Yes	****

Dunnett's multiple comparisons test (PE)	Mean Diff.	95% CI of diff.	Significant?	Summary
Negative vs. Sample	-5.833	-6.223 to -5.444	Yes	****
Negative vs. Positive	-12.00	-12.39 to -11.61	Yes	****

Table No. 5. Showing Antimicrobial Activity of '*Arkadigana*' Against *Pseudomonas aeruginosa*

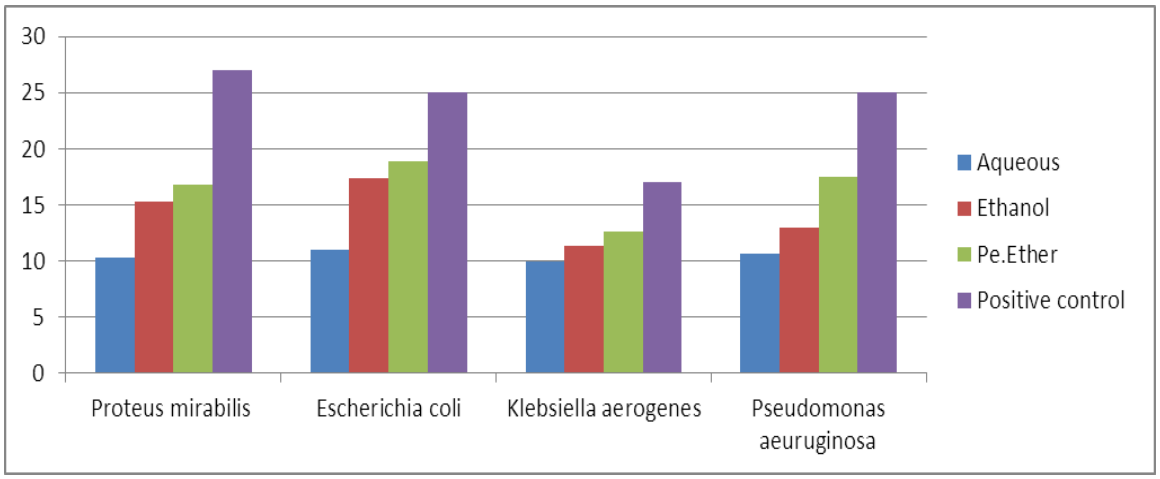
Pseudomonas Aeruginosa	NEGATIVE mean ± s.e.m.	SAMPLE mean ± s.e.m.	POSITIVE mean ± s.e.m.	P. VALUE mean ± s.e.m.	SIGNIFICANCE
Aqueous	0±0	10.33±0.0577	25±0		
Ethanol	12±0	13±0.0577	25±0	<0.0001	H.S.
Pt. Ether	13±0	16±0.02887	25±0	<0.0001	H.S.

Dunnett's multiple Comparisons test (PE)	Mean Diff.	95% CI of diff.	Significant?	Summary
Negative vs. sample	-3.833	-4.223 to -3.444	Yes	****
Negative vs. Positive	-14.00	-14.39 to -13.61	Yes	****

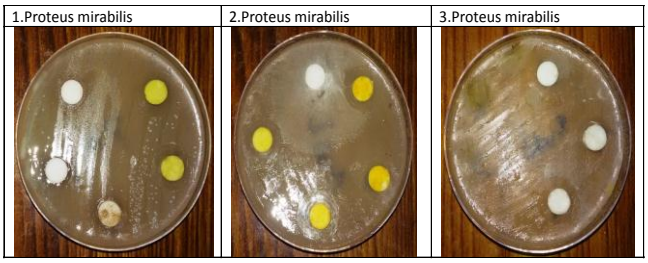
Table No. 6. Showing Antimicrobial Activity of '*Arkadigana*' Against *Klebsiella aerogenes*

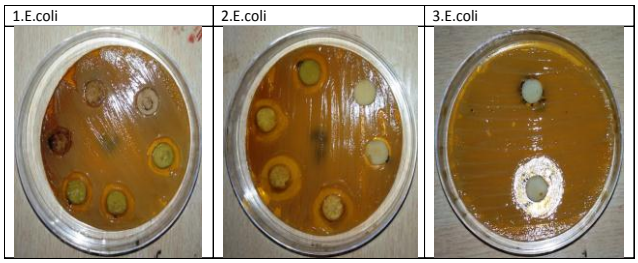
Klebsiella aerogenes	NEGATIVE mean ± s.e.m.	SAMPLE mean ± s.e.m.	POSITIVE mean ± s.e.m.	P. VALUE	SIGNIFICANCE
Aqueous	0±0	10.33±0.0577	17±0		
Ethanol	0±0	11.33±0.0577	17±0	<0.0001	H.S.
Pt. Ether	0±0	12.66±0.0577	17±0	<0.0001	H.S.

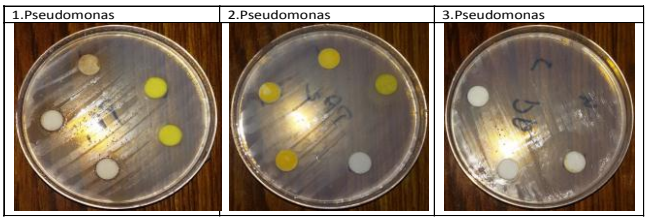
Graphical representation for zone of different extracts of *Arkadigana and* positive control against different microorganism (Disc diffusion method)



PHOTOS OF ANTIMICROBIAL STUDY







DISCUSSION

- Infections can run from one person to another under various kinds of personal contacts. Thus, *Acharya Sushruta* has enumerated the reasons for the transfer of infections, while dealing with the pathogenesis of '*Kustha'*. It is needless to say, epidemic diseases happen only due to communicability of germs. The theory of epidemics is deeply dealt within *Charaka Vimana Sthana* (Chapter3).
- For disinfections purpose, the various '*Rakshoghna'* drugs are used. And prevention of epidemics, *Charaka* has asked to keep a collection of medicinal herbs before the epidemics and to follow the daily and seasonal regimen. For treatment purpose, *Acharya Sushruta* has asked to perform *Puja*, *Homa*, *Havana,* etc. and gave advice to vacate the place where the epidemic is present.

- *Apakarsana*, *Prakrtivighata* And *Nidana-Parivarjana* are the principles of treatment mentioned in context to combat the parasitic infections.
- The anti-microbial activity incorporates *Visaghna*, *Vranaśodhana* and *Kleda-Puyopasosana* activities.
- *Acharya Sushruta* mentioned in *Sutrasthana* 38th chapter, example of *Kapha Saman Draya* are *Rasna, Ingudi, langali etc are* drugs are consider in *Arkadi gana*. So direct act on *Krimi*.
- *Shodhana* in this context refers to *Sroto-shodhana* and irrigation of the local debris by means of *Lekhana* action and *Laghu* and *Tikshna* properties of the *Arkadi gana*. This ultimately cleans the *Vrana*.
- At the end of *Shodhana Chikitsa*, *Vrana* becomes *Shuddha* and *Ropana Chikitsa* has to be followed further.
- Petroleum ether and ethanol extracts of the *Arkadigana*, 15.33 and 16.83 mm highly active compare to Vancomycin against *Proteus mirabilis*.
- Petroleum ether and ethanol extracts of the *Arkadigana*, 17.33 and 18.83 mm highly active compare to Vancomycin against *Escherichia coli*.
- Petroleum ether extracts of the *Arkadigana*, 12.66 mm active compare to Vancomycin against *Klebsiella aerogenes*.
- Petroleum ether extracts of the *Arkadigana*, 16 mm highly active compare to Vancomycin against Pseudomoans *aeruginosa*.

CONCLUSION

- From the study it can be concluded that '*Arkadi gana*' (herbal preparations) are strong antibacterial agent against all bacteria, these pathogens are responsible for wound infections, osteomyelitis and associated with dysentery, diarrhoea, gastroenteritis, urinary tract infection and skin infections.
- There are a good number of medicines available in *Ayurveda* for various diseases, but till now efforts have not been made to specifically find out what medicines should be used for what condition.
- It is now essential to confirm the action of drug on particular pathogenic micro-organisms by microbiological techniques so as to make our treatment scientifically more validated. This research work could be a small step towards this goal.

  Our study indicated that the aqueous extracts are strong antibacterial as compare to organic solvent extracts (petroleum ether and ethanol) against Proteus mirabilis, E.coli

and P. aeruginosa

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