EDUZONE: International Peer Reviewed/Refereed Multidisciplinary Journal (EIPRMJ), ISSN: 2319-5045
Volume 13, Issue 2, July-December, 2024, Available online at: www.eduzonejournal.com

Antimicrobial Activity of Tutthakadya Malahara- In Vitro Study

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ABSTRACT

Bhaishjya Kalpana is a field of Ayurved that deals with the pharmaceutical preparation of medicinal formulations. There are various types of formulations mentioned in Ayurvedic texts for the purpose of external application like Lepa, Upanaha, Pradeha, etc. Tutthakadya Malahara, one of such drugs, useful in the treatment of Vrana. Though, it is indicated in skin diseases; its Malahara form was found in classical text 'Rastarangini' as indicated in Vranashodhana and Vranaropana, but evidences of its use can be obtained through traditional practices. Analytical study of a drug helps to interpret the possible physical and chemical changes occurring during various pharmaceutical processing of the material.

Keywords- Malaharayogas, Tutthakadya Malahara, Microorganisms, Antimicrobial study, Vranashodhana, Vranaropan

INTRODUCTION

MARAHAMA (MALAHAM) is an Arabic word, meaning plaster, dressing for wounds. The synonym Lepa directly infers the link between Malahara Kalpana & Lepa Kalpana. During 8th A.D., both Charak and Sushruta Samhitas were translated into Arabic and Persian languages, which would have influenced the Unani System to the origin of Marahama Kalpana from Ayurvedic Lepa Kalpana. Malahara Kalpana comes under Bahya Kalpana (external application). The word 'Malahara' was adapted by Yogaratnakara[1] from the word Malaham or Marham which is originated from Unani system of medicine.[2] The word Malahara means that it removes Mala from Vrana, Vidradi, Twak Vikara etc. It is a quite widely used ointment preparation with many advantages. Malahara Kalpana differs from Ghrita, Taila and Upanaha Kalpana though looking similar they are also used for external application, but they are not necessarily semisolid (in all seasons) stable, smooth and soft. While Malahara Kalpana is not only applied externally, but is semisolid in all seasons, stable, smooth and soft. and Tutthakadya Malahara is a herbo-mineral formulation intended for external application in various wound-healing, using natural ingredients Goghruta as base for preparation. Hence, this study is undertaken to evaluate Pharmaceutico-Analytical study of Tutthakadya Malahara and its Antimicrobial Activity.

Aim

To evaluate pharmaceutico-analytical study of Tutthakadya Malahara and its antimicrobial activity

Objectives-

- Preparation of Tutthakadya Malahara as per the reference taken from Rasa Tarangini, Chapter 21, Verse 100-104.
- In vitro study of anti-microbial activity of Tutthakadya Malahara.

MATERIALS AND METHODS

The preparation of test drug Tutthakadya Malahara was carried out in the Pharmacy of Rasashastra and Bhaishajya Kalpana Department, D. Y. Patil, deemed to be University, school of Ayurved, Nerul, Navi Mumbai, and the In-vitro study was carried out in Biotech testing services, Ghatkopar, Mumbai.

PREPARATION OF TUTTHAKADYA MALAHARA[3,4,5,6]

घृतं व्दितोलकमितं दर्वीस्थं वन्हिना पचेत् ।
तोलकादष्टमांशं वा पादिकं रालकं क्षिपेत् ॥
रालन्तु विद्रुतं ज्ञात्वा द्रुतं खल्वे विनिक्षिपेत् ।
दत्त्वाथ निर्मलं तुत्थं तोलकस्याष्टमांशिकम् ॥

खटि कपर्दभसितं टङ्कःश्चैकैकतोलकम् ।
पषयेव्दारिणा तावन्मुञ्चन्नीरन्तु नीलताम् ॥
मुहुर्मुहुः क्षिपेन्नीरं मर्दनान्नील्ता गतम् ।
विशालस्ये काचकृपे निदध्यादथ यत्नतः ॥
तुत्थकाद्यो मलहरी व्रणशोधनः परम् । (R. T. 21/100-104)

Materials:

Sr. No.	Ingredients[2]	Quantity[2]	Weight
1.	**Go-Ghrita** (Clarified Butter)	2 Tola	24 gms
2.	**Raala** (Shorea Robust resin)	1/8 Tola or ¼ Tola	1.5gms/ 3 gms
3.	**Tuttha** (Blue Vitriol/ Copper sulphate, CuSo4)	1/8 Tola	1.5 gms
4.	**Kapardika Bhasma** (Marine shell or Cowrie)	1 Tola	12 gms
5.	**Khatika** (Chalk or Pipe Clay)	1 Tola	12 gms
6.	**Tankan** (Borax)	1 Tola	12 gms
7	**Water**	As per need	

Method:

1) All ingredients were weighed according to the mentioned proportion
2) Go-ghruta was melted over medium flame in a clean vessel, flame was put off once the Go-ghruta had melted
3) Raal (powder) was added to Go-ghurta and mixed properly
4) Shodhit Tankan, Shodhit Khatika, Shodhit Tuttha and Kapardika Bhasma were added and mixed properly
5) Water was added to this mixture and was washed thoroughly until the water turned blue.
6) The blue water was removed and fresh water was added and mixed again.
7) This process of changing water and rubbing thoroughly was repeated until clean water was obtained
8) The water was changed for a total of 25 times, to obtain clear water.
9) The obtained Malahara is stored in a sterile jar and labelled as Tutthakadya Malahara.

Duration: 6 hours
Initial Weight: 322 gms
Final weight of Tutthakadya Malahara: 355 gms
Weight Gain at the end of process: 33 gms

SOURCE OF CHEMICAL AND REAGENTS

All the chemical reagents and other requirements of the experimental study were used from the stock of biotech testing services.

OBSERVATIONS AND RESULTS OF EXPERIMENTAL STUDY

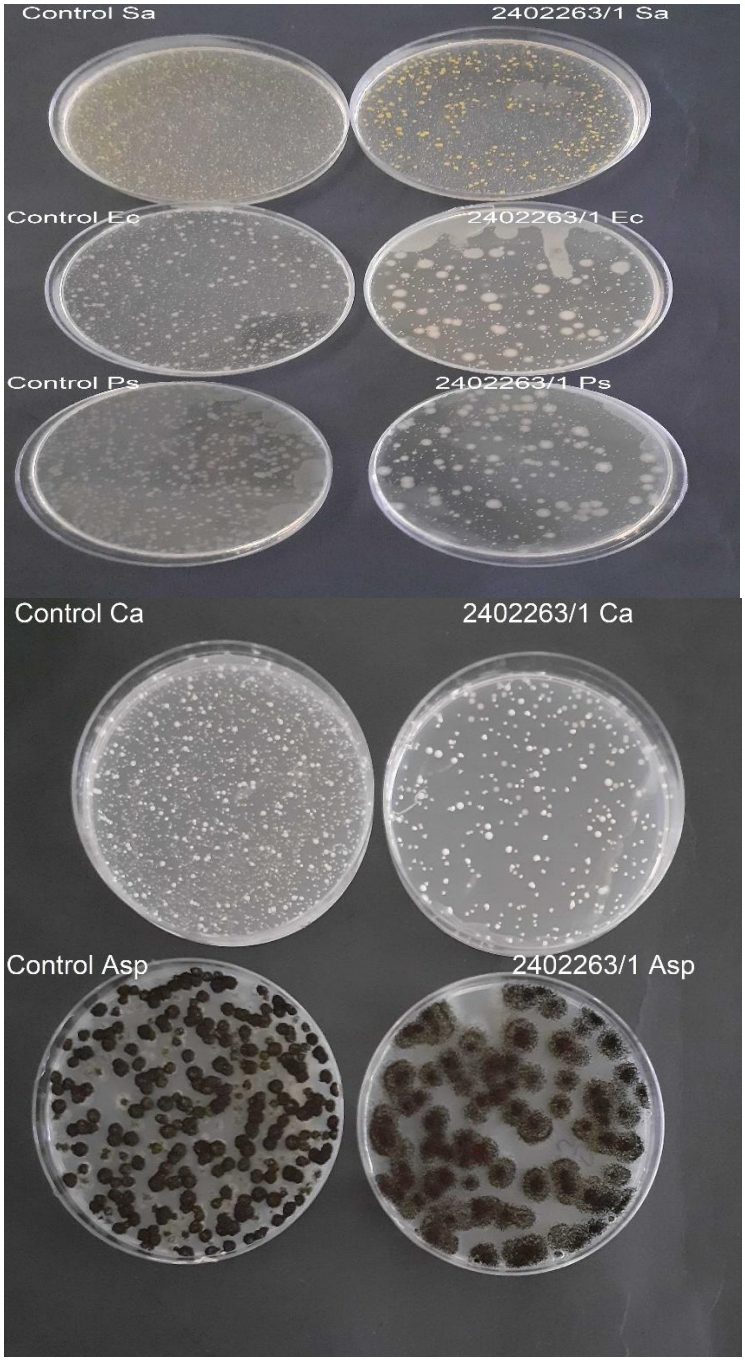
The Antimicrobial activity of Tutthakadya Malahara was studied. Before testing the antimicrobial activity, the sample was tested for its microbial limit.

SAMPLE DETAILS-

Tutthakadya Malahara (20µl)

TEST COMPOUND

Sample (100mg/ml)



In vitro antimicrobial study of Tutthakadya Malahara

RESULT

Result of Antimicrobial Control Test (Time Kill Procedure)

Name of Test :
ASTM E 2315 - 16
Assessment of Antimicrobial Activity Using a Time-Kill Procedure
Test Organisms :

1. Staphylococcus aureus ATCC 6538
2. Escherichia coli ATCC 10536
3. Pseudomonas aeruginosa ATCC 15442
4. Candida albicans ATCC 10231
5. Aspergillus niger ATCC 6275

Test Procedure:
The product was inoculated with test organisms individually (approximately 10^6 CFU/ ml). After the specified exposure time, the surviving microorganisms were recovered by drawing an aliquot, neutralizing it and performing the Standard Pour plate Technique. Culture count was ascertained by dilution Blank. Adequate Validation of Neutralizing agent was also carried out. Test was carried out in duplicate and average count was taken as CFU/ ml.

Experimental Conditions

Test Product	: Ready to use
Neutralizer	: DE broth
Contact Time	: 5 minutes
Contact Temperature	: 20°C ± 1°C
Growth Media	: Soyabean-casein digest agar at 37°C for 48 hours Sabourauds Dextrose agar at 28°C for 72 hours
Incubation Condition	: 37°C for 48 hours

Neutralizer Validation:

Validation Controls			
Test Organism	Experimental Conditions Control (A)	Neutralization Toxicity Control (B)	Neutralization Efficacy Control (C)
Staphylococcus aureus ATCC 6538	50 µg/ml	51	52
Escherichia coli ATCC 10536	60	62	61
Pseudomonas aeruginosa ATCC 15442	54	58	56
Candida albicans ATCC 10231	32	36	34
Aspergillus niger ATCC 6275	28	30	31

RESULTS

Sample Description	Test Organism	Exposure Time	Microbial Count		Antimicrobial Log Reduction	Antimicrobial Percentage Reduction
			Count	Log		
2402263/S01 Sample-1 (RTU)	**ɔhylococcus aureus ATCC 6538**	Initial	1.70×10^5	5.23	-	-
		5 minutes	1.50×10^4	4.17	1.05	91.17
	Escherichia coli ATCC 10536	Initial	1.80×10^5	5.25	-	-
		5 minutes	2.20×10^4	4.34	0.91	87.77
	lomonas aeruginosa ATCC 15442	Initial	1.52×10^5	5.18	-	-
		5 minutes	1.80×10^4	4.25	0.92	88.15
	ida albicans ATCC 10231	Initial	1.45×10^5	5.16	-	-

		5 minutes	1.54 x 10	4.18	0.97	89.37
	Aspergillus niger ATCC 6275	Initial	1.28×10^5	5.10	-	-
		5 minutes	1.40×10^4	4.14	0.96	89.06
Percentage Reduction of Microorganism = 100 (Initial Microbial count - Microbial count after Exposure)/ Initial Log Reduction = Log Initial Microbial count - Log Microbial count after Exposure						

Results of Microbial Limit Test:

Microbiological Enumeration Tests			
Sl. No.	**Test Parameter**	**Test Method**	**Test Observation**
1	Total Plate Count	USP 39; Chapter <61>: 2021	<10 CFU/gm
2	Total Yeast & Mould Count	USP 39; Chapter <61>: 2021	<10 CFU/gm

DISCUSSION[7-12]

- Skin is the largest organ of the body with a total area of 20 square feet. It plays a vital role in acting as an anatomical barrier between pathogens and our body. It also helps in thermoregulation and permits the sensation of touch, heat and cold. Any disruption in the continuity of skin can allow the dirt particles and pathogens to enter the body thus causing infections. Ayurved, being a science with vast knowledge that has a lot to offer to the humankind, has mentioned many formulations for problems associated with skin but most of these treatment modalities are not been validated with modern research methodology.
- Tutthakadya Malahara is a herbomineral formulation mentioned in the text of Rasa Tarangini and is indicated in Vrana Chikitsa (Wound Management). The preparation of Tutthakadya Malahara was carried out according to the procedure mentioned in Rasa Taranagini keeping in mind the need for a standard method of preparation, an effort was made to follow the hygiene protocols and use of authentic and good quality raw material.
- The colour of final product was grey, during the initial stage, when Raal was melted in Go-ghruta, the colour was dark brown, like that of the Raal, but once all other ingredients were added and then water was added and the process of rubbing was carried out, the malahara began to turn grey in colour.
- The process of adding water, rubbing the Malahara and then changing the water had to be repeated until the water stopped turning blue. It took 25 washes (25 times of water change) for the water to stop turning blue. This process helped in forming a homogeneous mixture and also increased the skin penetration rate producing good effects. The application of Tutthakadya Malahara was done on dorsal aspect of hand to assess the smoothness of the Malahara.
- The action of a drug mainly depends on the properties of the raw materials used and hence a detailed study of the pharmacodynamics of the ingredients and their properties is very important before starting with the pharmaceutical study.
- Ingredients used in the Malahara like Tankan, Khatika, Kapadrika Bhasma are said to have twachya property (helpful for skin) and also inhibit the property of Vrana Ropana (Wound healing). Other ingredients like Tuttha have Krimighna (Anti-microbial) property and Goghruta is the best media used, as it presents as a good moisturizer for the skin and also has the ability to inhibit the properties of any drug that it is mixed with it. Raal acts as a binding agent, giving the Malahara the desired smoothness.
- The pharmaceutical and therapeutic properties of a formulation are majorly influenced by its physical and chemical properties. These physico-chemical properties of a formulation decide its therapeutic action and its effect and utility and hence the physico-chemical study of any drug or formulation play a vital role in understanding about the drug/formulation. The effect or action of any formulation is a combined effect of the physico-chemical characters of its ingredients.
- The organoleptic test gives a detailed knowledge about the drug and makes its identification and authentication easier. Tutthakadya Malahara was a semisolid preparation (Sparsha), its Roop was grey with small black spots, Rasa was Acrid and Gandha was characteristic of Go-ghruta.
- Other physico-chemical test like pH, Loss on Drying, Spreadability, Thermal Stability were also carried out on the Malahara sample.
- pH of Tutthakadya Malahara was 5.0% which is considered to be slightly acidic, this property of the Malahara can help it inhibit the ‘Puyanissarak’ Property as mentioned in the reference shloka. Tutthakadya Malahara was

smooth with greyish colour. Loss on Drying gives a measure of the moisture content and the sample of Tutthakadya Malahara showed 7.15% moisture content. The sample was found to be uniformly spreadable thus proving it to be easy for application.

- The Malahara sample was tested for its thermal stability and it was observed to be stable till 45°C, proving its stability parameter under intense heat. Rancidity refers to the chemical decomposition of fats, oils and other lipids which can cause a foul smell of the product which can make it unpleasant to use. Rancidity test for the Tutthakadya Malahara Sample was found to be negative. It is important for a formulation containing more than one ingredient to be a homogeneous mixture so that all the ingredients are uniformly distributed along the entire sample. The content uniformity of the Tutthakadya Malahara sample was found to be equal throughout the entire sample.

CONCLUSION

Tutthakadya Malahara is mentioned in Rasatarangini, which is a combination of five ingredients. Identification, collection of the drugs and preparation of Tutthakadya Malahara was done as per the classical reference. A experimental study was conducted to evaluate the antimicrobial activity and cytotoxic effects of Tutthakadya Malhara in wound healing. The study concluded that:

- Drugs which are used for the preparation of Tutthakadya Malahara viz., Tuttha, Goghruta, Khatita, Kapardika Bhasma, Raal and Tankan have Vrana Shodhan and Vrana Ropana property and are effective in wound healing.
- The pH of Tutthakadya Malahara is found to be acidic and it gently helps in cleansing the wound.
- The Malahara sample was tested for its thermal stability and it was observed to be stable till 45°C, proving its stability parameter under intense heat.
- The sample was found to be uniformly spreadable thus proving it to be easy for application.
- pH of Tutthakadya Malahara was 5.0% which is considered to be slightly acidic, this property of the malahara can help it inhibit the ‘Puyanissarak’ Property as mentioned in the reference shloka. Tutthakadya Malahara
- The formulation demonstrated mild antimicrobial activity against organisms such as Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, and Aspergillus niger.

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