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Research Article

PHARMACEUTICAL DEVELOPMENT & ANTI-MICROBIAL STUDY (IN VITRO) OF *TANKANAMRUTA MALAHAR* W.S.R. TO RASA TARANGINI

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ABSTRACT

"Tankan" which is among the *'Kshar traya'* mentioned in *"Kshar trik vidnyaniya trayodasha taranaga"* of Acharya Sadananda Sharma virachit 'Rasa tarangini'. *Tankan* i.e., Borax ($Na_2B_4O_7$ $10H_2O$) is composed of boric acid & soda. It is a salt of tetra Boric acid, an important compound of Boron, which is also known as sodium biborate. Other *Rasa Vaidyas* also have included *"Tankan"* under the groups of *Ksharatraya, Ksharapanchaka, Dravaka gana, Mitra panchaka* etc. The present study was conducted to develop *"Tankanamruta malahar"* (pharmaceutically & to study its antimicrobial activity (In vitro). *"Tankanamruta malahar"* is a good combination of herbo-mineral preparation, which is indicated in *Dushta vrana shodhan chikitsa* or infected wounds. Though this formulation is from Ayurvedic classical text but there is a need to develop the standard procedure of preparation as they have faster & accurate results than oral administration of drug, so by the fusion of modern instruments & traditional methods of manufacturing procedures we have tried to make *"Tankanamruta malahar"* which contains *Shuddha Tankan bhasma, Siktha taila, Sarji kshar, Shuddha pushpa kasis* & *Pipal twak kshar*. Standardization describes all measures taken during manufacturing process like *Shodhan, Maran, Kshar nirman* & *Siktha taila* preparation etc & quality control leads to reproducible quality of particular product & in vitro study was performed to evaluate its anti-microbial activity against selected pathogens.

KEYWORDS: *Tankan, Tankanamruta malahar, Rasa tarangini*, Borax, *Dushta vrana*.

INTRODUCTION

Ayurveda refers to the traditional health & healing system of India. Since the ancient time it was believed that Ayurvedic herbs & minerals are supposed to give a solution for all kinds of diseases which is even considered impossible by other field of medical science. Our ancient Acharyas were mainly involved in experimenting the different kinds of herbs & minerals then the preparation of Ayurvedic medicine from them.

"Tankanamruta malahar" is also an important herbo - mineral formulation which is described in *Acharya Sadananda Sharma virachit 'Rasa tarangini'* 13/96-99 for *Dushta Vrana Chikitsa.*[1] This formulation is the ideal combination of various methods described in Rasashastra & Bhaishajya Kalpana. For preparation of this *Malahar*, we have to perform *Siktha taila* preparation, *Shodhan & Bhasmikaran* of selected ingredients, *Kshar nirman* & finally *Malahar kalpana.*

As allopathic drugs have more resistance to pathogens causing wound infections, Ayurveda is a science of medicine where there are so many pieces of pearl available to treat wound without any complication. Though *"Tankanamruta malahar"* is a textual reference there is a need to develop the standard procedure of preparation as they have faster & accurate results than allopathic drug without any complications.

We have done this study in 2 parts & they are – pharmaceutical development & standardization of "*Tankanamruta malahar*" & to assess its antimicrobial activity against *Staphylococcus aureus* (ATCC NO. 6538) which is Gram +ve bacteria & *Pseudomonas aeruginosa* (ATCC NO. 9025) which is Gram –ve bacteria because they both are more resistant to the allopathic drugs. All the ingredients of this *Malahar* are having *Vrana ropak, Shothaghna, Krimighna, Vishaghna, Kledak* properties. Therefore, the current study is aimed to develop a safe, potent, effective & convenient to use formula with the longer shelf life & cost effective therapeutic strategy of Ayurveda for infected wounds in the form of *"Tankanamruta malahar".*

AIM & OBJECTIVES

AIM

Pharmaceutical development of the standard manufacturing process of *Tankanamruta malahar &* study of its antimicrobial activity (in vitro).

OBJECTIVES

1. Pharmaceutical development & standardization of *Tankanamruta malahar*.
2. To analyze the study drug physico-chemically.
3. To screen the antimicrobial activity (in-vitro) of the study drug to analyze the results.

MATERIALS & METHODS

MATERIALS

"Tankanamruta malahar" is a good combination of herbo-mineral preparation. As per the reference given in the *Ras tarangini*, it contains *Shuddha Tankan bhasma, Siktha taila, Sarji kshar, Shuddha pushpa kasis* & *Pipal twak kshar*. All the required raw material was collected from Local flower market, Dadar, Mumbai & All India Kirana Stores (Raw Material Drugs) Shop, Pydhonie, Mumbai & RJK International Chemical manufacturer, Kalbadevi, Mumbai & authenticated from the Dravyaguna & Rasashastra & Bhaishajya kalpana departments of Y.M.T. Ayurvedic Medical College, Kharghar, Navi Mumbai.

Table 1: Place Of The Study

Pharmaceutical & analytical study	In vitro study
Y.M.T. Ayurvedic Medical College & hospital, Kharghar, Navi Mumbai	Shraddha Analytical Services, Ghatkopar (W), Mumbai.

Table 2: Ingredients For *Tankanamruta Malahar* (Ref: *Rasa Tarangini* 13/96-99)

Sr. No.	Ingredients	Quantity
1	*Shuddha tankana bhasma*	*2 tola* = 24 gm
2	*Shuddha pushpa kasis*	*½ tola* = 6 gm
3	*Sarji kshar*	*½ tola* = 6 gm
4	*Pipal twaka kshar*	*2 masha* = 2 gm
5	*Siktha taila*	*12 tola* = 144 gm

Table 3: The drugs identified are tabulated as in following table

Sr. No.	Name of drugs	Common name	Action	Other specifications
1	*Shuddha Tankan*	Borax	*Vishaghna, Vrana nashaka, Kaphaghna, Lekhaniya, Varnya*	***Rasa*** *– Kshariya* ***Guna*** *– Ruksha, Tikshna, Guru* ***Virya –*** *Ushna*
2	*Shuddha Kasis*	Green Vitriol	*Kandughna, Vishaghna, Vrana ropaka*	***Rasa*** *– Amla, Tikta, Kashaya* ***Guna*** *– Grahi, Ushna* ***Virya*** *– Ushna* ***Vipaka*** *– Katu*
3	*Sarji Kshar*	Mixture of Potassium Salts	*Vrana ropaka, Shothaghna, Krimighna Kaphaghna, Daha nashak, Kledaka*	***Rasa*** *– Katu,* ***Guna*** *– Ruksha, Tikshna, Laghu* ***Virya*** *– Ushna*
4	*Pipal Twaka Kshar*	Salt of *Ficus religiosa* bark	*Vranaropaka, Shothaghna, Krimighna*	***Rasa*** *– Katu, Kshariya* ***Guna*** *– Ruksha, Tikshna* ***Virya*** *– Ushna*
5	*Siktha Taila*	Bee's wax oil	*Kledaka*	

Table 4: Batches of *Tankanamruta Malahar* for Standardization

S. No.	Ingredients	TM – 1	TM – 2	TM – 3
1	*Shuddha tankana bhasma*	24 gm	24 gm	24 gm
2	*Shuddha pushpa kasis*	6 gm	6 gm	6 gm
3	*Sarji kshar*	6 gm	6 gm	6 gm
4	*Pipal twaka kshar*	2 gm	2 gm	2 gm
5	*Siktha taila*	144 gm	144 gm	144 gm

Instruments & Equipment

Table 5: For *Tankan Shodhan & Bhasmikaran*

Stainless steel vessel	Spoon	Khalva yantra

Table 6: For *Kasis Shodhan*

Dola yantra	Khalva yantra	Spoon
Gas stove with cylinder & lighter	Cotton cloth	Flat plate for drying

Table 7: For *Pipal Twaka Kshar Nirman*

Gas stove with cylinder & lighter	Stainless steel vessel with spoon	Sieve
Weighing balance	Cotton cloth	Measuring cylinder

Table 8: For *Siktha Taila Nirman*

Gas stove with cylinder & lighter	Stainless steel vessel	Spoon
Measuring cylinder	Weighing balance	Container for storage

Table 9: For *Tankanamruta Malahar Nirman*

Stainless steel vessel	Spoon	Container for storage

Method I) Pharmaceutical Method

Presentation of *Tankan Shodhan & Bhasmikaran*

1. *Ashuddha Tankan* was taken in a clean & dry *Khalva yantra* & pounded well to prepare *Churna* (powder) of it.
2. This *Churna* (powder) was taken in to an iron vessel & was heated on *Mandagni* (Mild heat), followed by *Tivragni* (maximum heat).
3. Continuous stirring was done of *Tankan churna* (powder), until all the water content in it was completely evaporated
4. Finally *Shuddha Tankan* is obtained as a white coloured puffy light substance.
5. *Shuddha tankana* was trichurated until it became fine *Bhasma*.

Fig. 1: Schematic Presentation of *Tankan Shodhan & Bhasmikaran*

Presentation of *Kasis Shodhan*

1. *Bhringraj swaras* was obtained from fresh *Bhringraj* for *Shodhan* of *Ashuddha kasis*.
2. *Ashuddha kasis* was lightly pounded in *Khalva yantra* & *Pottali* was made from it.
3. *Ashuddha Kasis* was purified in *Bhringraj Swaras* in *Dola yantra* for 3 *Ghatika* (72 min).

Fig. 2 Schematic Presentation of *Kasis Shodhan*

Presentation of *Pipal Twaka Kshar Nirman*

1. *Pipal Twaka* was dried under the sun light & these dry *Twaka* was burnt into ashes.
2. After completion of burning, the ash was allowed to cool down
3. *Pipal Twaka* ash was dissolved in 6 parts of water, stirred well & kept undisturbed overnight.
4. Next morning the supernatant water was filtered for 21 times called as *Ksharodaka*.
5. The filtrate - *Ksharodaka* was boiled in open large iron vessel with constant slow stirring on mild to medium flame.
6. When the boiling liquid turns brown, slimy, clear & pungent smelling, it was removed from the fire & allowed to settle.
7. *Ksharodaka* was further boiled to get it in paste or dry powder, known as *Pipal Twaka Kshar*.

Fig. 3: Schematic Presentation of *Pipal Twaka Kshar Nirman*

Presentation of *Siktha Taila Nirman*

1. 6 part of *Til taila* (Sesame oil) was heated over mild fire, until foam starts appearing.
2. 1 part of *Siktha* – Bee's wax was added to it.
3. After the wax was completely melted in oil, it was filtered & allowed to cool while continuous stirring.
4. *Siktha taila* was ready & stored properly, for preparing *Tankanamruta malahar*.

Fig. 4: Schematic Presentation of *Siktha Taila Nirman*

Presentation of *Tankanamruta Malahar Nirman*

1. 144 gm of *Siktha Taila* was taken into clean & dry stainless steel vessel.
2. 24 gm of *Shuddha Tankan Bhasma* was added to it.
3. It was vigorously stirred by the palms, until *Shuddha Tankan Bhasma* gets mixed properly (10 min).
4. Then after 6 gm of each *Sarji Kshar & Shuddha Pushpa Kasis* was added & it was stirred about 10 -15 min.
5. 2 gm of *Pipal Twaka Kshar* was added & the mixture was stirred vigorously for about 15-20 min.
6. After sufficient stirring a homogenous mixture of *Tankanamruta Malahar* was prepared, stored it in appropriate containers & labeled. The process is repeated for 3 times to prepare 3 batches for standardization purpose.

Fig. 5: Schematic Presentation of *Tankanamruta Malahar Nirman*

For the purpose of standardization 3 batches were prepared of *Tankanamruta malahar*. They were named as TM-1, TM-2 & TM-3 respectively. (TM – *Tankanamruta malahar*)

II) In Vitro Study

The study material was divided into 2 groups

I) *Tankanamruta malahar*

II) Soframycin cream – Framycetin sulphate

Table 15: Micro organisms

S.No.	Genus	Species	Kingdom	Atcc No.
1	*Staphylococcus*	*Staphylococcus aureus* (Gram +ve)	Eubacteria	ATCC 6538
2	*Pseudomonas*	*Pseudomonas aeruginosa* (Gram –ve)	Bacteria	ATCC 9025

The experimental study (In vitro) will be conducted at Shraddha Analytical Services, Ghatkopar, Mumbai. The following methods will be carried out during the study.

Plan of Antimicrobial Study (In Vitro) Study

A. Steps

1. Procurement of cultures of microorganisms from culture collection centre.
2. Preparation of antimicrobial study materials (test samples) as mentioned above.
3. Evaluation of antimicrobial activity (in vitro).

B. Microbial Assay: Antimicrobial activities of groups of formulations against pathogenic microorganisms will be evaluated by using Agar disc diffusion method – for zone of inhibition & plate dilution method –for Minimum inhibitory concentration (MIC).

C. Method

I) Minimum Bactericidal Concentration (MIC) by Plate Method - ASM: Manual of Microbiology methods.

II) Antimicrobial Property of test by Disc Diffusion Method as per NCCLS guidelines 2005.

Minimum Bactericidal Concentration (MIC) by Plate Method - ASM: Manual of Microbiology methods

Purpose

The minimum Bactericidal concentration (MBC) of an antibacterial is the maximum dilution of the product that kills the growth of a test microorganism. In plate dilution method, serial dilutions of the products are made in bacterial growth media. The test organisms are then streaked on the surface of the same, incubated, & scored for growth.

Antimicrobial Property of test by Disc Diffusion Method as per NCCLS guidelines 2005

Test Procedure

The test organisms diluted to approximately 10^7-10^8CFU/ ml was individually spread by a sterile swab evenly over the face of Soyabean Casein digest agar. Test preparation equivalent to 30 ul was smeared on sterile disc. Thus disc containing test preparation was then placed on seeded plate. Control plate comprised of distilled water solution on disc. The plates incubated at 37˚C for 24 hrs. Zone of inhibition were measured by calibrated ruler. Zone of inhibition signifies the presence of antibacterial activity. Higher the zone of inhibition, greater is the diffusible antibacterial activity. No zone of inhibition & growth on & around disc indicate no antibacterial activity.

Observation

Physico-Chemical Analysis of *Tankanamruta Malahar*

A. *Panchabhautika Parikshana (Shabda, Sparsha, Roop, Rasa, Gandha)*

B. Modern Parameters

- **Physical appearance -** The *Tankanamruta malahar* was evaluated in terms of physical character like change in colour, odour & rheological parameters.
- **pH value -** The pH of the *Tankanamruta malahar* was determined by using digital pH meter i.e., electrode method and pH strip method. 500 mg of drug was taken & dissolved in 50 mL distilled water & measurement of pH was done in triplicate & average value was calculated.
- **Homogeneity -** *Tankanamruta malahar* was tested for homogeneity by visual inspection after it was kept to set in the container. It was tested for appearance & presence of any aggregates.
- **Skin irritation test -** Test for irritation was performed on human volunteers with their consent. Five volunteers were selected & 1.0 g of *Tankanamruta malahar* was applied on an area of 2 square inch to the back of hand. The volunteers were observed for lesions or irritation.

❖ **Rancidity test (Kreis test) –** The test depends upon the formation of a red colour when oxidized fat is treated with conc. HCl & a solution of phloroglucinol in ether. The compound in rancid fats responsible for the colour reaction is epihydrin aldehyde. All oxidized fats respond to the Kreis test & the intensity of the colour produced is roughly proportional to the degree of oxidative rancidity. Mix 1 ml of melted fat & 1 ml of conc. HCl in a test tube. Add 1 ml of a 1 % solution of phloroglucinol in diethyl ether & mix thoroughly with the fat-acid mixture. A pink colour formation indicates that the fat is slightly oxidized while a red colour indicates that the fat is definitely oxidized.

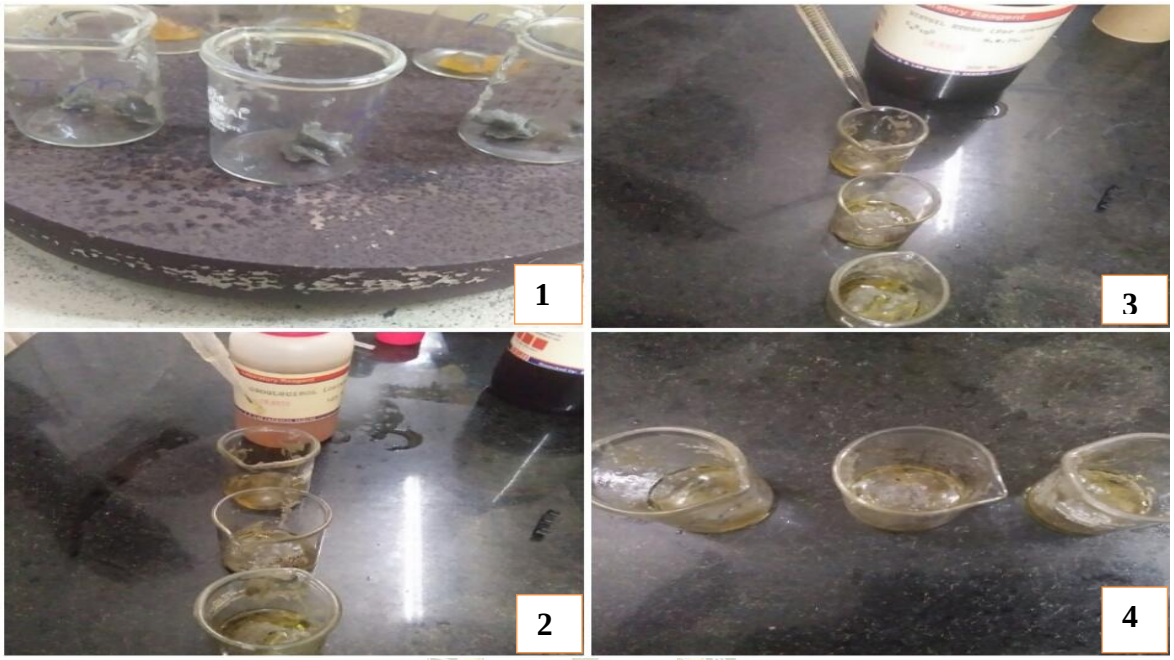


Fig. 6: The study was repeated three times for standardization purpose

Table 10: *Panchabhautika Parikshana (Shabda, Sparsha, Roop, Rasa, Gandha)*

Pariksha	**Batch – 1 (TM – 1)**	**Batch – 2 (TM – 2)**	**Batch – 3 (TM – 3)**
Shabda	No specific sound	No specific sound	No specific sound
Sparsha	*Snigdha – Picchila*	*Snigdha – Picchila*	*Snigdha – Picchila*
Roop	Greyish coloured homogenous mixture	Greyish coloured homogenous mixture	Greyish coloured homogenous mixture
Rasa	Not applicable	Not applicable	Not applicable
Gandha	Characteristic smell	Characteristic smell	Characteristic smell

Table 11: Modern Parameters

Modern parameters	**TM – 1**	**TM – 2**	**TM – 3**
Physical appearance	Grey coloured homogenous mixture	Grey coloured homogenous mixture	Grey coloured homogenous mixture
pH value	8.33 (By strip – 8)	8.35 (By strip – 8)	8.26 (By strip – 8)
Homogeneity	Homogeneous, smooth, oily & slimy consistent malahar. No aggregates found.	Homogeneous, smooth, oily & slimy consistent malahar. No aggregates found	Homogeneous, smooth, oily & slimy consistent malahar. No aggregates found
Skin irritation test	Skin compatible. No irritation found	Skin compatible. No irritation found	Skin compatible. No irritation found
Rancidity test	No colour change. No Rancidity seen.	No colour change. No Rancidity seen.	No colour change. No Rancidity seen.

Minimum Bactericidal Concentration (MIC) by Plate Method – ASM: Manual of Microbiology methods

Table 12: Concentration of MIC: 0.1%, 1%, 2%

Test product	Test organisms	Concentration of test product			MIC (In %)
		0.1%	1%	2%	
Tankanamruta malahar	*Staphylococcus aureus*	Growth	No Growth	No Growth	1%
	Pseudomonas aeruginosa	Growth	No Growth	No Growth	1%
Soframycin (Framysetin sulphate)	*Staphylococcus aureus*	Growth	No Growth	No Growth	1%
	Pseudomonas aeruginosa	Growth	No Growth	No Growth	1%

Note:

Positive Control comprised of Media + Organisms; Growth observed for Test bacteria

Negative Control comprised of Medium Blank: No Growth

Fig. 7 Fig. 8

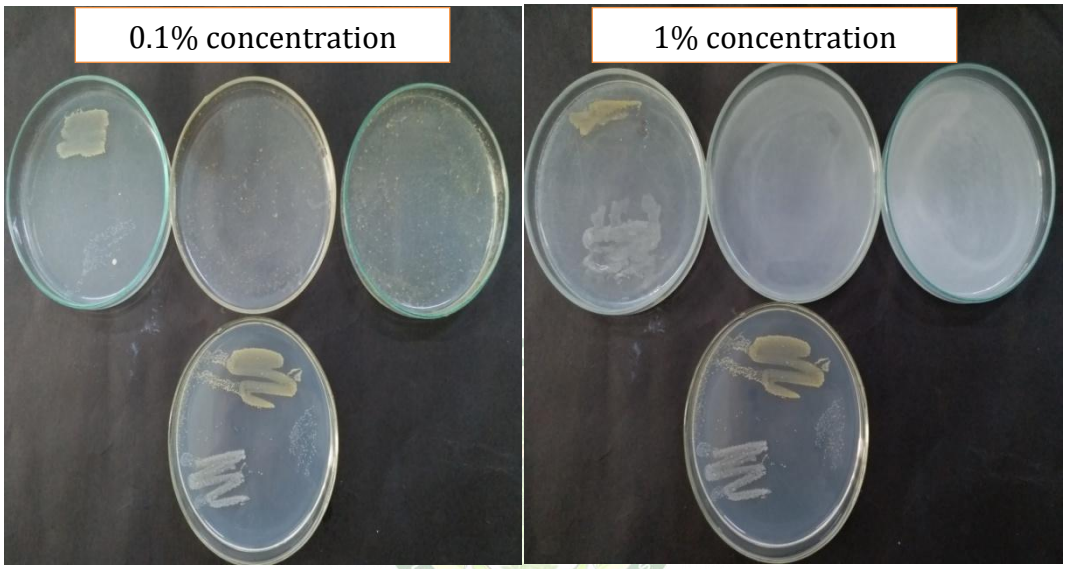
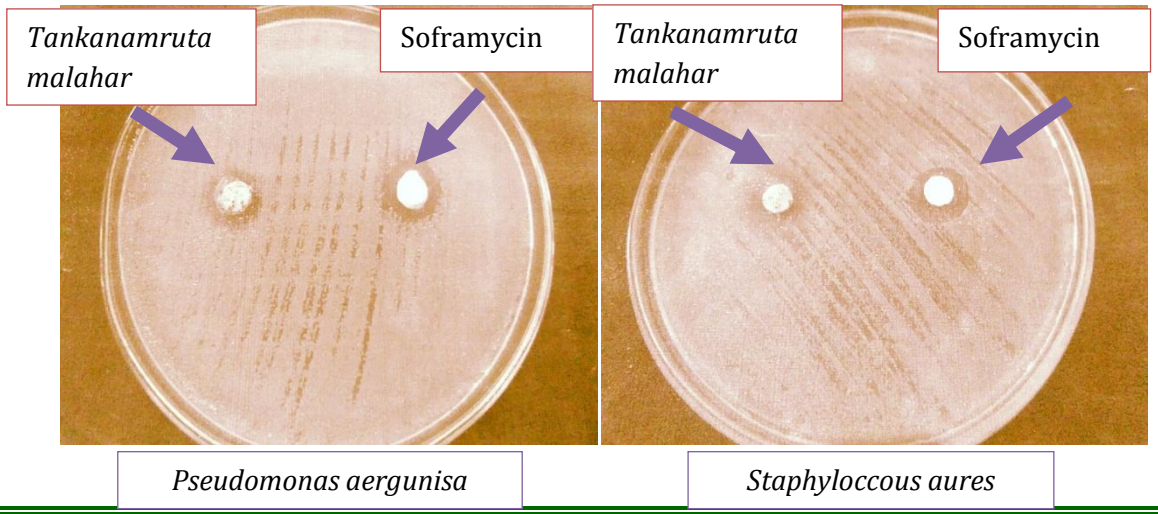


Table 13: Antimicrobial Property of test by Disc Diffusion Method as per NCCLS guidelines 2005

Test sample	Zone of inhibition in mms	
	Staphylococcus aureus	***Pseudomonas aeruginosa***
Tankanamruta malahar	12 mm	12 mm
Soframycin (Framycetin sulphate)	12 mm	13 mm

Note:

1. 30 ul of test preparation was used per disc.
2. Larger the zone more is the concentration of antibacterial substance.



Result & Discussion

A. Pharmaceutical standardization

Reports of physical appearance, pH, homogeneity, skin irritation test & rancidity test are given in above table. An average value of pH of formulated *Tankanamruta malahar* was around 8.31, which is alkaline in nature. A good viscosity is required to have an acceptable formulation, too viscous formulation may cause pourability problem whereas too low viscosity may cause settling of dispersed contents while storage. The *Malahar* was moderate viscous in appearance. Application of a *Tankanamruta malahar* is comfortable if the base spreads easily, exhibiting maximum slip & drag. This *Malahar* produces good spreadability. The physical appearance of the *Tankanamruta malahar* in batches – 1, 2 &3 were grey coloured homogenous mixtures. All the 3 batches were homogenous, smooth, slimy & consistent with no aggregates or sediments with skin compatible & no irritation found on application & no colour change & no rancidity was seen.

B. Anti-microbial activity

The present study deals with the preliminary screening & comparison of Antimicrobial activity of *Tankanamruta malahar* & Soframycin. Both the study drugs have been tested against *Staphylococcus aureus* & *Pseudomonas aeruginosa* for antimicrobial activity. The antimicrobial activity of *Tankanamruta malahar* & Soframycin was equal in 1% concentration against both the organisms by Minimum Bactericidal Concentration (MIC) by Plate Method – ASM: Manual of Microbiology methods.

Zone of inhibition was also calculated in mms for *Tankanamruta malahar* & Soframycin by Antimicrobial Property of test by Disc Diffusion Method as per NCCLS guidelines 2005. Zone of inhibition of *Tankanamruta malahar* & Soframycin was seen equal against *Staphylococcus aureus* & it was about 12mm. Zone of inhibition of *Tankanamruta malahar* against *Pseudomonas aeruginosa* was 12mm & 13mm for Soframycin, which is just 1 mm larger than *Tankanamruta malahar.*

Shuddha Tankan is used in the process of repair that follows injury to the skin & other soft tissues. The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, & scarring to the patient.

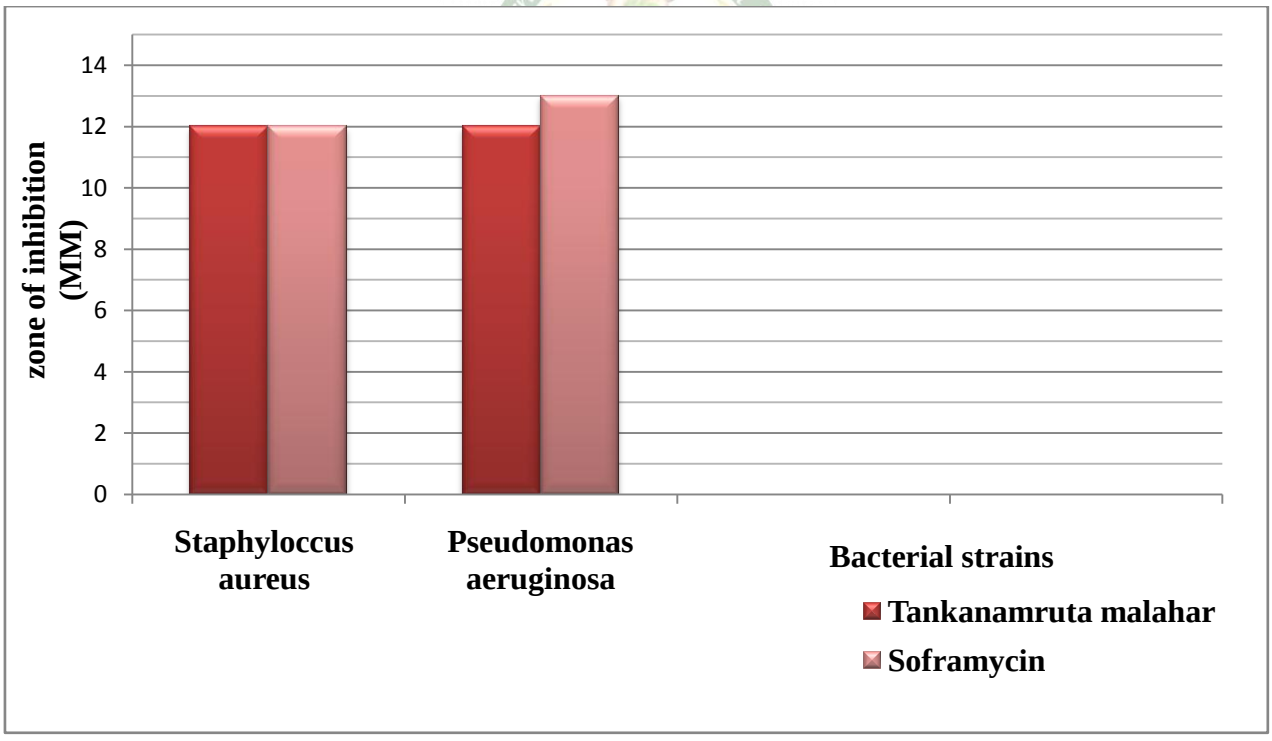


Fig. 11 Anti-microbial activity

CONCLUSION

From the present study, we can conclude that *Tankanamruta malahar* was formulated & standardized successfully as per the reference given in the *Rasa tarangini* 13/96-99 & showed significant antimicrobial activity against *Staphylococcus aureus (*Gram +ve) & *Pseudomonas aeruginosa* (Gram –ve). This topical application may have minimum side effects as compared to the allopathic drugs. *Tankanamruta malahar* may have more active & potent herbo – mineral ingredients because it is free from any added preservatives like allopathic drugs.

As *Tankanamruta malahar* is indicated in *Dushta vrana chikitsa* (Infected wounds) [2], hence we have selected such 2 bacteria for this experimental study which are more resistant to allopathic antibiotics. As Ayurvedic topical applications have minimum side effects than allopathic, this *Malahar kalpana* is found to be more beneficial in terms of physico-chemical parameters& antimicrobial activity.

Tankan is used in the process of repair that follows injury to the skin & other soft tissues [4-6]. The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, & scarring to the patient. All the ingredients of *Tankanamruta malahar* have *Vrana ropaka, Shothaghna, Krimighna, Kandughna, vishaghna, Kledaka* properties hence, all these collectively will provide more benefits to the wound healing process. As anti-bacterial activity of *Tankanamruta malahar* is proved from this study pre clinically against *Staphylococcus aureus* (Gram +ve) & *Pseudomonas aeruginosa* (Gram –ve), now there is a need to design a clinical trial to check its efficacy on different kinds of wound infections & to assess its wound healing action on human volunteers or by in vivo study.

Finally we can conclude that Ayurvedic management gives equally or more superiorly satisfactory results than allopathic drugs in terms of potency, shelf life, cost efficacy & action etc. Still there are so many potent herbo-mineral formulations are there described by our ancient Acharyas which requires to be standardized & to bring it in daily practice. This study is an attempt to bring one of them in front of the people from ancient texts to daily routine practice.

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