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PHARMACEUTICO – ANALYTICAL STUDY OF *VISARPAHARA TAILA* AND ITS ANTIMOCROBIAL ACTIVITY

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ABSTRACT

Visarpahara Taila is an Ayurvedic formulation which is mentioned in *Rasatantrasara Va Siddhaprayogasangraha* for the management of *Visarpa Roga.* In Ayurvedic classics the *Visarpa* is not a single entity of disease. The common feature of all the types is its quick spreading nature, based on this factor *Visarpa* can be correlated with many fast-spreading infectious diseases such as erysipelas, cellulitis etc. As it can be co related to many diseases which are caused by various microorganisms. This study was conducted with an aim to assess the antimicrobial effect of *Visarpahara Taila* against a select few bacteria which are responsible for common skin diseases.

KEYWORDS: *Visarpahara Taila, Rasatantrasara Va Siddhaprayogasangraha,* Antimicrobial activity, Agar well diffusion method, Kirby-Bauer disc diffusion method, Analytical study.

INTRODUCTION

In Ayurveda various formulations such as solid dosage forms (*Vati, Churna* etc), liquid dosage forms (*Taila, Asava, Arishta* etc) and semisolid dosage forms (*Ghrita, Avaleha* etc) are used for therapeutic purposes. Among all these formulations *Taila Kalpana* is one of the most important *Kalpana*, as this form is used for all the four modes of drug administration like *Pana, Abhyanga, Nasya* and *Basti. Visarpahara Taila*[1] is one such formulation which is

mentioned in *Rasatantrasara Va Siddhaprayogasangraha* for the management of *Visarpa Roga.* It is having clinical features such as *Sphota, Shopha* etc.[2] In modern science *Visarpa* is usually correlated with Herpes / Erysipelas. It is a mucocutaneous infection caused by Herpes simplex virus.[3] In Ayurvedic classics *Visarpa* is mentioned to be one such disease that spreads easily and quickly in various directions. It is not a single entity of disease, all the skin infections having quick spreading nature comes under it. Hence it can be correlated to many fast-spreading infectious diseases. Based on these factors the bacteria which are most commonly present in skin infections were selected for anti-microbial study of *Visarpahara Taila.*

AIM AND OBJECTIVE

Aim: To understand the effect of *Visarpahara Taila* on most commonly found bacteria.

Objectives of the study

- To prepare *Visarpahara Taila* as per classical method.
- To do physico-chemical analysis of *Visarpahara Taila.*
- To evaluate the antimicrobial property of *Visarpahara Taila*.

Hypothesis

- H_0 – *Visarpahara Taila* is not having antimicrobial property.
- H_1 – *Visarpahara Taila* is having antimicrobial property.

MATERIAL AND METHODS

Pharmaceutical Study

Table No 1: Ingredients as well as quantity used for *Taila* preparation.

DRUG	BOTANICAL NAME	PART USED	QUANTITY MENTIONED	QUANTITY TAKEN
Paribhadra	*Erythrina indica* Lam.	Leaves, Bark, Root	1 Part	200 g
Narikela Taila	*Cocos nucifera* Linn.	Fruit	4 Parts	800ml
Jala	-	-	16 parts	3200ml

All the ingredients were procured from the Alva's Pharmacy, Mijar, Karnataka and authenticated by the experts of Alva's Atma Research Centre, Moodubidire Dakshina Kannada Karnataka. All the data were found strictly as per guidelines.

Equipments: Measuring jar, Weighing machine, Utensils, Fire source, Cloth, Bottle, Spatula, *Khalwa Yantra***.**

Method of Preparation

Visarpahara Taila was prepared according to the classical reference. This includes following steps.

Preparation of *Kalka Dravya*

Equal quantity of *Paribhadra* leaves, bark and root were collected, weighed and pounded separately into coarse powder. It was later mixed together and made into bolus.

Preparation of *Taila Kalpana*

Day 1

Narikela Taila was taken in a clean stainless-steel vessel and heated on a mild flame to remove the moisture content. The bolus of *Kalka* was slowly added to it with constant stirring, 16 parts of *Jala* was added and mixed thoroughly to form a homogeneous mixture. The heating was continued over *Mandagni* with continuous stirring, for proper mixing. Once it had reached the boiling state, the stove was turned off and the vessel was covered with a cloth, tied and left overnight.

Day 2

Next day the heating process of *Taila* was continued in *Madhyama Agni* with constant stirring. This procedure was done until it had attained *Sneha Siddhi Lakashanas*. After reaching *Sneha Siddhi Lakshanas*, the heating was stopped and *Taila* was filtered immediately through a clean cloth. The filtered *Taila* was measured and stored in air tight container.

OBSERVATIONS AND RESULT

- The prepared *Kalka* seemed to be bulky, as root and bark of the *Paribhadra* was fibrous which probably increased the bulk.
- On second day the heating was restarted, the *Kalka* was seen to be floating over the surface of the oil. The heating process was continued for 4hrs after which appearance of *Taila Paka Siddhi Lakshana* were seen.
- The *Taila Paka Siddhi* were assessed based on following observation

1. Frothy appearance of *Taila* which indicated the beginning of *Paka Lakshana*
2. Absence of crackling sound when the oil was exposed to fire.
3. *Kalka* could be made into wick on rolling it between the fingers.
4. Separation of *Kalka* from *Taila* was seen.

The oil was filtered and *Kalka* was separated, the obtained oil was light green in color. Quantity of oil obtained was 500 ml and there was a loss of 300 ml, as the *Kalka* was bulky in nature and it had absorbed most of the oil which might have resulted in the loss.

Photographs showing the preparation of *Visarpahara Taila*

Fig 1 Ingredients

Fig 2 Ingredients added to the vessel

Fig 3 Heated over medium flame

Fig 4 Separation of *Kalka* from *Taila*

Fig 5 Filtering of Taila

Fig 6 Final product

ANALYTICAL STUDY

The Physico-chemical analysis of *Visarpahara Taila* was carried out in the Quality Control Lab of Sri Dharmasthala Manjunatheshwara centre for research in Ayurveda and Allied science Udupi, Karnataka. The following result was obtained.

Table no 2: Results of standardization parameters.

Parameter	***Results n = 3 %w/w***
	Visarpahara Taila
Refractive index	1.45232
Specific gravity	0.9066
Viscosity ($kgm^{-1}s^{-1}$)	44.97
Acid value	4.67
Saponification	226.34

value	
Iodine value	20.30
Peroxide value	0.38
Rancidity	Fat is not oxidized

EXPERIMENTAL STUDY

IN-VITRO ANTIMICROBIAL STUDY

This study was done with an aim to evaluate antimicrobial activity of the *Visarpahara Taila* against a select few bacteria which are responsible for common skin diseases. It was assessed by following two methods that is.

- Agar well diffusion method[4]
- Kirby Bauer disc diffusion method.[5]

The In -Vitro antimicrobial study was done in Medical Microbiology Laboratory, Alvas college of Allied Health Sciences Moodubidire.

MATERIAL AND METHOD

At first only *Visarpahara Taila* was used as a test sample but it had failed to diffuse in the Muller- Hinton Agar media. Seeing this, further modifications were done by trial and error method. The final study was conducted by adding a reagent Tween 20 to the test sample.

Practical no. 1.

Test organism: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Sample: *Visarpahara Taila.*

AGAR WELL DIFFUSION METHOD[4]

Materials Required: Mueller Hinton agar plate, Incubator, Borer, Pipette, Laminar air flow, Swab**,** Test tube**,** Spirit lamp**,** Vortex Mixer**,** Hot plate**,** Beaker. Etc.

Reagent: Tween 20 (Polysorbate 20).

Preparation of inoculums

A loopful of each organism were inoculated into peptone water and incubated at 37℃ for 4 to 6 hours till light to moderate turbidity appears. If turbidity in the broth is sufficient further incubation is not necessary.

Preparation of Samples

A test tube was taken and added with 5 ml of *Visarpahara Taila* to which 1.250 ml (20%)of tween 20 was added. They were warmed at 50℃ by using double boiling method, that is a glass beaker was filled with water and placed over hot plate and heated until the it reaches 50℃. Once it reaches the required temperature the test tubes containing the samples were placed in it for 20 minutes.

Agar well diffusion method: Mueller-Hinton agar plates were prepared, the medium in the plate should be sterile and having a depth of 4mm. A sterile non-toxic cotton swab was dipped in the respective peptone water culture of the test bacteria. This moistened cotton swab was streaked on the entire Mueller –Hinton agar surface of the plate 3 times, turning the plate at 60^0 angle between each streaking. The inoculum was allowed to dry for 5 to 15 minutes with lid in place. A cork borer (4mm) was used to bore a well in the media and test sample of *Visrpahara Taila,* was dispensed into the well by using a micropipette. The commercially available Amikacin antibiotic disc was also placed to compare the antibiotic sensitivity in each plate. The plates were incubated at 37℃ for 24 hours. The zone of inhibition was measured at the end of incubation.

OBSERVATIONS AND RESULTS

After 17 hrs of incubation a clear zone of inhibition was seen in the bacteria *Staphylococcus aureus* (15 mm), whereas in *Pseudomonas aureuginosa* (15mm) a clear zone of inhibition was seen after 24 hrs of incubation. The zone of inhibition of *Klebsiella pneumoniae* (14mm) and *Escherichia coli* (15mm) was not as clear as that of the other species of bacteria, but *Escherichia coli* had larger zone inhibition than *Klebsiella pneumoniae*.

Table No 3: Zone of inhibition of bacterias as seen in Agar well diffusion method.

Sl no	Name of the Bacteria	Zone of inhibition of standard drug	Zone of inhibition of test drug
1	*Staphylococcus aureus*	20 mm	15mm
2	*Pseudomonas aeruginosa*	20mm	15mm
3	*Escherichia coli*	20mm	15mm
4	*Klebsiella pneumoniae*	20mm	14mm

KIRBY-BAUER DISC DIFFUSION METHOD[5]

Materials Required: Mueller Hinton agar plate**,** Incubator**,** Whatman No 1 filter paper**,** Pipette, Laminar air flow**,** Swab**,** Test tube**,** Spirit lamp**,** Hot plate**,** Vortex mixer**,** Beaker etc.

Reagent: Tween 20 (Polysorbate 20 solution).

Preparation of inoculums

A loopful of each organism was inoculated into peptone water and incubated at 37℃ for 4 to 6 hours till light to moderate turbidity appears. If turbidity in the broth is sufficient further incubation is not necessary.

Preparation of Samples

A test tube was taken and added with 5 ml of *Visarpahara Taila* to which 1.250 ml (20%) of tween 20 was added. They were warmed at 50℃ by using double boiling method, that is a glass beaker was filled with water and placed over hot plate and heated until the it reaches 50℃. Once it reaches the required temperature the test tubes containing the samples were placed in it for 20 minutes.

Preparation of the disc

Sterile 4mm diameter Whatman No 1 filter paper discs was soaked in the test sample *Visarpaha Taila* for 6 hrs.

Disc diffusion method: Mueller-Hinton agar plates were prepared, the medium in the plate should be sterile and have a depth of 4mm. A sterile non- toxic cotton swab was dipped in the respective peptone water culture of the test bacteria. This moistened cotton swab was streaked on the entire Muller –Hinton agar surface of the plate 3 times, turning the plate at 60^0 angle between each streaking. The inoculum was allowed to dry for 5 to 15 minutes with lid in place. The soaked discs were applied using aseptic technique on the surface of Mueller-Hinton agar plate. The commercially available Amikacin antibiotic disc were also placed to compare the antibiotic sensitivity in each plate. The plates were incubated at 37 ℃ degree for 24 hours. The zone of inhibition was measured at the end of incubation.

OBSERVATIONS AND RESULTS

Staphylococcus aureus and *Pseudomonas aeruginosa* showed a clear zone of inhibition after 24 hrs of incubation. Zone of inhibition was absent in *Klebsiella pneumoniae* and *Escherichia Coli* after 24 hrs. of incubation.

Table No 4: Zone of inhibition of bacterias as seen in Kirby- Bauer disc diffusion method.

Sl.No	Name of the Bacteria	Zone of inhibition of standard drug	Zone of inhibition of test drug
1	*Staphylococcus aureus*	20 mm	12mm
2	*Pseudomonas aeruginosa*	20mm	12mm
3	*Escherichia coli*	20mm	No zone of inhibition
4	*Klebsiella pneumoniae*	20mm	No zone of inhibition

Photographs showing the results of Agar well diffusion method

Fig 7 *Staphylococcus aureus*

Fig 8 *Pseudomonas aeruginosa*

Fig 9 *Escherichia coli*

Fig 10 *Klebisella pneumoniae*

Photographs showing the results of Kirby- Bauer disc diffusion

Fig 11 *Staphylococcus aureus*

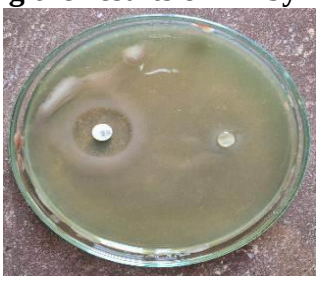

Fig 12 *Pseudomonas aeruginosa*

Fig 13 *Escherichia coli*

Fig 14 *Klebisella pneumoniae*

DISCUSSION

The *Visarpahara Taila* was prepared according to the classical method, where 1 part of *Kalka* was added to 4 parts *Taila* along with 16 parts of *Drava Dravya* and heated. The *Jala* was used as the *Drava Dravya* here, as the specific liquid was not mentioned. The *Kalka* was prepared by adding little quantity of water as the drugs used here were dry in nature. *Taila* was priorly heated before the addition of *Kalka* in order to remove the moisture content. The *Taila Paka* was completed within 2 days by following the reference of *Sharangadhara Samhitha* as keeping it overnight potentiates the *Taila* preparation. The heating was continued the next day until it had attained *Madhyama Paka*. There was a gradual change in the colour

which may be due to the changes occurring in the ingredients of the *Taila* during the *Paka*. It can also be taken as an indication of solubility of more active principle into the *Taila* with the increased contact time. The *Taila* was filtered immediately through the cloth in order to prevent loss, as the *Kalka* might reabsorb the oil on cooling. The *Taila* was green in colour, this may due to the presence of chlorophyll content from the *Paribhadra* leaves. The obtained oil was 500 ml, there was a loss of 300 ml, this may be due to the bulky nature of the *Kalka*. As the *Kalka* was dry and fibrous in nature, it had absorbed maximum amount of oil during the preparation of *Taila.* This loss can be rectified without decreasing its potency, by reducing the amount of *Kalka* and by using *Paribhadra Kashaya* (prepared from the same ingredients as that of *Kalka*) instead of *Jala.*

Standardization of a formulation is an important procedure that has to be done in order to bring uniformity in the prepared formulations, with respect to chemical and biological parameters. It helps in a ensuring the quality and purity of the material used during the preparation of the medicine. Hence analytical study of *Visarpahara Taila* was carried out, which can be used as a base for further studies.

The antimicrobial study was first carried out by using only oil as the test sample, but there was no zone of inhibition seen. It may be due to the fact that oil is viscous and is known to be immiscible in nature, hence it had failed to diffuse in agar medium. To counteract this, further studies were carried out by mixing the test sample with the reagent tween 20 in various proportions. 20% of tween 20 showed maximum dispersion of oil in agar medium hence this proportion was used in the final study. 20 % of tween 20 was added to the test sample and the mixture was warmed in double boiling method, this was done based on the principle that the viscosity of the oil decreases with the increase in temperature. The temperature was set up to 50℃ for 20 min as the tween has a tendency to breakdown at higher temperature. The test was carried out by pipetting the test sample into the wells in Agar well diffusion method whereas in Kirby Bauer disc diffusion method the discs were allowed to be soaked in in the sample for 6hrs prior to its application on the agar medium this was done in order to let the disc to absorb the maximum amount of oil.

The result obtained were, the bacteria *Staphylococcus aureus* showed a clear zone of after 17 hours of incubation. This shows that the bacteria have high sensitivity towards the test sample *Visarpahara Taila. Pseudomonas aeruginosa* showed a clear zone of inhabitation towards the test sample after 24 hrs of incubation, this shows that it has lesser sensitivity towards the test

sample when compared with *Staphylococcus aureus. Klebsiella pneumoniae* and *Esherichia coli* showed a lighter zone of inhibition when compared with the rest of the bacterias , this shows that their sensitivity towards the test sample was quite low. *Escherichia coli* showed a bigger zone compared to *Klebsiella pneumoniae*; this shows that *Escherichia coli* has higher sensitivity towards *Visarpahara Taila* when compared with *Klebsiella pneumonia.*

In Kirby – Bauer disc diffusion method *Staphylococcus aureus* showed a clear zone of inhibition after 17 hrs of incubation whereas *Pseudomonas aeruginosa* showed a lighter zone of inhibition after 24 hrs of incubation. The zone of inhibitions was seen to be lesser than that of previous study, this might be due to the fact that the discs may not have absorbed sufficient amount of *Taila. Escherichia Coli* and *Klebsiella Pneumoniae* showed no zone of inhibition it may be due the lesser sensitivity of the bacterias towards the *Visarpahara Taila.* Hence it might need larger quantity of oil than that absorbed by the discs to show its sensitivity.By seeing the above results, we can conclude that Kirby-Bauer disc method is not suitable for antimicrobial study on *Sneha Kalpanas.*

CONCLUSION

The Pharmaceutico- analytical study of *Visarpahara Taila* as well as its antimicrobial activity on some select bacteria was carried out here. This study had helped us to obtain a standard analytical parameter of *Visarpahara Taila* which can used as a base for further studies. The antimicrobial study had revealed that the bacteria's such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* showed varying degree of sensitivity towards *Visarpahara Taila*. Hence the null hypothesis was rejected and alternate hypothesis that is *Visarpahara Taila* is having antimicrobial activity was accepted.

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