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FORMULATION, ANTI BACTERIAL ACTIVITY AND WOUND HEALING PROPERTY OF *PANCHAVALKALADI* OINTMENT

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ABSTRACT

Preventing secondary infections in the surgical wounds with safe and efficacious anti bacterial agents is must in the current scenario to enhance the wound healing process. The purpose of the present study was to prepare an *Ayurvedic* ointment comprising of *Panchavalkala* and *Triphala*, the known herbal combinations with multi dimensional activities and to evaluate the anti bacterial activity and to screen its wound healing property. Disc diffusion method was adopted for anti bacterial activity and the results showed the ointment to be bioactive. Screening of wound healing activity showed that the *Panchavalkaladi* ointment enhanced the process of wound healing.

Keywords: *Panchavalkala, Triphala, Ayurveda,* ointment, wound healing

INTRODUCTION

The global acceptance of *Ayurveda* has raised the responsibility of *Ayurvedic* pharmaceutics to design the dosage forms which are user friendly. The days when *Vaidya* used to prepare and dispense the medicines by himself do no longer exist. Many pharmaceutical companies have come up in the market with new dosage forms to substitute the age old *Vati, Gutika, lepa* etc. It's the need of the hour to use the modern advanced pharmaceutical technologies and upgrade the *Ayurvedic* medicines. *Lepa* is one such *Ayurvedic* medicines which can be modified into latest ointment form to make it user friendly. With this intention an *Ayurvedic* ointment was formulated consisting of *Panchavalkala* (barks of *Ficus bangalensis* Linn, *Ficus gloerata* Roxb, *Ficus religiosa* Linn, *Thespesia populnea* Soland and *Ficus lecor* Buch) and *Triphala* (fruits of *Terminalia chebula* Retz, *Terminalia belerica* Roxb, and *Phyllanthus emblica* Linn) which have multi dimensional activities.

Panchavalkala has activities like *vranaprakshalana, vranaropana, shothahara, upadanshahara*, *visarpahara*[1] and *Triphala* posseses anti inflammatory, analgesic, anti-arthritic, hypoglycemic and anti-aging properties.[2]

Infection is a major problem in the management of wounds. Even though the development of synthetic antimicrobial agents persists, drug resistance and toxicity hinder their way. Various research works have been carried out to evaluate the anti microbial activity of herbal drugs groups separately but in this study both the groups i.e *Panchavalkala* and *Triphala* were used

together in formulating an ointment and evaluation of anti bacterial activity and wound healing property was assessed.

MATERIALS AND METHODS

Raw Materials: Barks of *Panchavalkala* and dried fruits of *Triphala* were procured from Pharmacy, National Institute of Ayurveda, Jaipur. Coarse powder of the drugs were made and used for the preparation of the ointment.

Preparation of *Kwatha* (Decoction): Coarse powder (sieve no. 10) of *Panchavalkala* and *Triphala* were prepared and soaked in 8 times of water for overnight. Next day it was subjected to heat with continuous stirring and the quantity was reduced to 1/4th of the initial volume. The liquid was filtered through four folded clean cotton cloth and the filtrate was collected as *Kwatha*. (Table 1)

Table 1: Composition of *Panchavalkaladi Kwatha*

Drugs	Latin Names	Part used	Proportion
Vata	*Ficus bengalensis Linn.*	Bark	70g
Udumbara	*Ficus glomerata Roxb.*	Bark	70g
Ashwattha	*Ficus religiosa Linn.*	Bark	70g
Parisha	*Thespesia populnea Soland.*	Bark	70g
Plaksha	*Ficus lecor Buch.*	Bark	70g
Haritaki	*Terminalia chebula* Retz	Fruit	50g
Vibhitaki	*Terminalia belerica* Roxb	Fruit	50g
Amalaki	*Phyllanthus emblica* Linn	Fruit	50g
Water	--	--	8 times Reduced to 1/4th

Preparation of *Ghana* (Solid extract): The *kwatha* obtained (1 liter) was subjected to mild heat till it becomes semi solid in consistency and the remnant (100g) was used as *Ghana* for the preparation of ointment.

Preparation of Ointment: Ointment was prepared with the concentration of 20% drug. Base was prepared with bees wax (50g) and petroleum jelly (350g). Initially bees wax was melted on water bath at 70^{o}c. Then petroleum jelly was added with continuous stirring to form a homogenous mixture. This mixture was then added with *Panchavalkaladi Ghana* and mixed properly to obtain chocolate/brown colored ointment. It was then stored in air tight containers. (Table 2)

Table 2: Composition of *Panchavalkaladi* ointment

Ingredients	Quantity
Panchavalkaladi Ghana	100g
Bees wax	50g
Petroleum Jelly	350g
Preservatives	0.1%

Preservatives: Methyl paraben and propyl paraben were used in the ration of 0.1%.

Anti Bacterial activity:

Agar Diffusion method: In current study the anti bacterial activity of the trial drug was carried out by agar diffusion method. The response of organisms to the trial drug was measured and compared with the response of the standard reference drug.

Streptomycin (5mg w/v) was the standard reference drug used in the study.

Micro organisms: Anti bacterial activity of the trial drug was evaluated in the below mentioned specified organisms:

1. *Escherichia coli* (gram negative)
2. *Staphylococcus aureus* (gram positive)
3. *Streptococcus pyogenes* (gram positive)

Standard Inoculum: Standardization of the inoculums is essential to provide reproducible MICs (Minimum inhibitory Concentrations). It is determined by comparing the turbidity of the liquid medium to a standard that represents a known number of bacteria in suspension. Here inoculums prepared by comparing with McFarland standards.

Culture medium: Mueller Hinton Agar

Composition: Beef infusion (300g/lit), Casein acid hydrolysate (17.5g/lit), Starch (1.5g/lit) and Agar (17g/lit). The final pH at 25^{o}c was maintained 7.3±0.1

Inoculation procedure: within 15 min after adjusting the turbidity of the inoculum suspension, dip a sterile cotton swab into the suspension. Pressing firmly against the inside wall of the tube just above the fluid level, rotate the swab to remove the excess liquid. Streak the swab over the entire surface of the medium three times, rotating the plate approximately 60 degree after each application to ensure an even distribution of the inoculums. Finally swab all around the edge of the agar surface.

Antibacterial disks: The working supply of antibacterial disks should be stored in the refrigerator (4^{o}c). Upon removal of the disks from the refrigerator, the package containing the cartridges should be left unopened at room temperature for approximately 1 hour to allow the temperature to equilibrate. This reduces the amount of condensation on the disks. Apply the antibacterial disks to the plates as soon as possible, but no longer than 15 min after inoculation. Place the disks individually with sterile forceps or with a mechanical dispensing apparatus and then gently press down on to the agar. Diffusion of the drug in the disk begins immediately; therefore once a disk contacts the agar surface the disk should not be moved.

RESULTS

Antibacterial sensitivity was performed for sample *Panchavalkaladi* ointment on Mueller Hinton against *E. coli*, *S. aureus* and *S. pyogenes* by well diffusion method; following were the results obtained using streptomycin 5mg w/v as positive control.

Table 3: Results of Anti bacterial activity

Bacteria	Positive control zone of inhibition (mm)	Sample zone of inhibition (mm)			Mean
		1	2	3	
E. coli	30.4	16	16.5	15	~16
S. aureus	44	19	19.5	19	~19
S. pyogenes	35.6	17	17.5	18	~18

Result Interpretation:

If mean value of the zone of inhibition is **<13** then it is considered as **Inactive**

If mean value of the zone of inhibition is **13-18** then it is considered as **Bioactive**

If mean value of the zone of inhibition is **>18** then it is considered as **Highly Active**

Wound Healing Study:

A pilot study of ten patients of post operative wound (cases of fistula in ano and pilonidal sinus) was conducted in OPD and IPD cases of *Shalya tantra*, NIA, Jaipur. The cases of healthy wound were selected and sterile dressing was done using

Panchavalkaladi ointment for a period of six weeks.

The study revealed that the test drug is potent in relieving pain and burning sensation. The wound remained healthy and healing process was promoted. By this study we can infer that *Panchavalkaladi* ointment has a role in relieving pain and burning sensation, which can be further justified with large sample study.

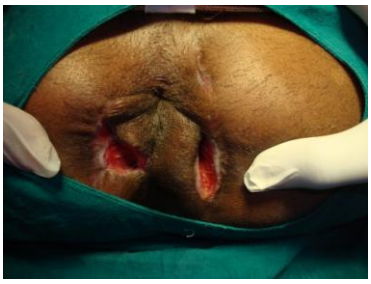

Fig 1: Fresh wound of Ischiorectal abscess

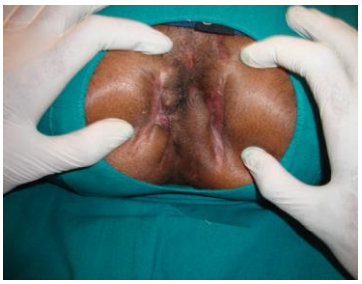

Fig 1A: After 6 weeks

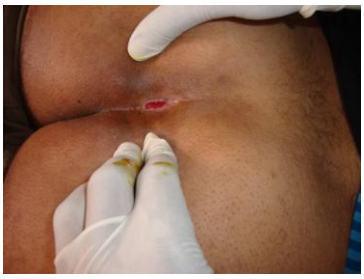

Fig 2: Pilonidal Sinus

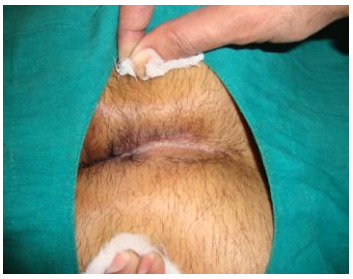

Fig 2A: After 6 weeks

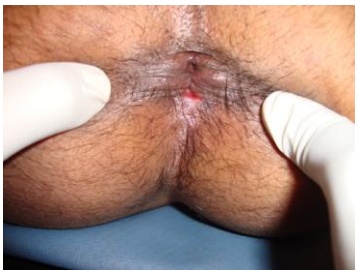

Fig 3: Fistulotomy Wound

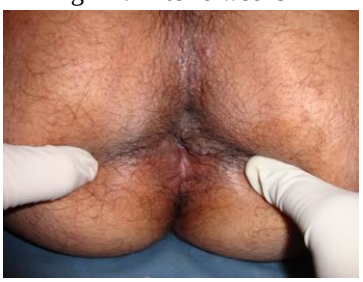

Fig 3A: After 6 weeks

DISCUSSION

There is no direct reference to this formulation but as these both groups of *Panchavalkala* and *Triphala* are known drugs which have anti inflammatory and anti microbial activity, they were mixed together and an *Ayurvedic* ointment was formulated. *Panchavalkala* was used 350g and *Triphala* was used 150g in the study based on the *Yukti*. Other ratio can also be tested in thid regard to test whether it will be more potent.

As *E. coli*, *S. aureus* and *S. pyogenes* are the most common organisms for secondary infections in surgical wounds; they were selected for the study. The results were no doubt less significant than the standard drug used in the study. But the standard drug streptomycin has its own side effects and limitations. Hence as the *Panchavalkaladi* ointment is herbal based and effective anti microbial and enhances wound healing, it can be a drug of choice in the management of post operative wound management. Further research can be continued with larger sample to justify these observations.

CONCLUSION

Panchavalkaladi ointment comprising of *Panchavalkala* and *Triphala* is an innovative formulation which is user friendly and acts as strong antibacterial agent in post operative surgical wounds and also enhances the wound healing process in the same.

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