Effect of Patolanimbapatra Arka Impregnated Dressing on Wound Microbiota and Healing in Surgical Wounds of Fissure-in-Ano – A Pre and Post Test Clinical Study

Dr. Aswathy A[1*], Dr. Sreedevi V[2], Dr. Rajeshwari P.N[3]

[1*]Assistant Professor, Department of Shalyatantra, SDM Trust's Ayurvedic Medical College, Terdal, Bagalkot, Karnataka, India, draswathyasok@gmail.com
[2]Assistant professor, Department of Shalyatantra, Amrita School of Ayurveda, Amritapuri, Kollam, Kerala, India, itz.devinair@gmail.com
[3]Professor, Department of Shalyatantra, Amrita School of Ayurveda, Amritapuri, Kollam, Kerala, India, dr.rajeshwaripn@ay.amrita.edu

[*]**Corresponding Author**
Dr. Aswathy A, Assistant Professor, Department of Shalyatantra, SDM Trust's Ayurvedic Medical College, Terdal, Bagalkot, Karnataka, India, draswathyasok@gmail.com
Contact number – 8157953981

ABSTRACT

Purpose: After surgery, bacteria quickly colonize open wounds in the skin. The microorganisms that colonize these wounds are usually either part of the patient's normal flora or may have been spread by contact with contaminated objects like water, dirt, faecal matter or healthcare personnel's hands. Infections in the wounds make it more difficult to heal after surgery and raise the expense of wound care considerably. Effective wound management depends on the development of new and useful ideas for the treatment and prevention of these wound infections.

Method: This study was carried out on 20 patients in Amrita School of Ayurveda's OPD and IPD. Individual drugs were collected and authenticated. *Paṭolanimbapatra Arka* was prepared as per *Arka kalpana,* and GC-MS of *Arka* was performed. The interventions were given to all participants once daily for 28 days.

Result: The prepared Arka's GC-MS analysis report suggests the presence of phytocompounds which is having proven antimicrobial, anti-inflammatory and anti-oxidant properties. All participants showed statistically significant improvement in outcome measures like Microbial Load, Presence of Pathogenic Organism, Size, Depth, Edge, Exudate Type, Exudate Amount, Skin Colour Surrounding Wound, Granulation Tissue, Epithelialization, Volume and Percentage of Healing. Peripheral tissue oedema and Peripheral tissue induration was hardly observed in 2 patients before treatment and it got reduced after 7 days. No adverse event was reported during the course of intervention.

Conclusion: Thus, it came to light that the *Paṭolanimbapatra Arka* impregnated gauze dressing was beneficial for wound microbiota and healing in the surgical wound of Fissure-in-Ano, which is susceptible to faecal contamination and infection.

Keywords: Āyurveda; Wound microbiota; Surgical wound of Fissure-in-Ano; Wound healing.

1. Introduction

Āyurveda, an age-old science has a huge treasure of classical formulations and methods for proper wound care and its management. Wound healing is a major concern in the surgical field because it needs to heal uneventfully. The goal of management is to create a hygienic atmosphere that deters infection and speeds up the healing of wounds. "Wound is a break in the integrity of the skin or tissues often, which may be associated with disruption of the structure and function".[1] It is thought that microorganisms are a major factor in poor wound healing and the emergence of infection-related complications[2]. Infection is another possibility with surgical wounds if they are not properly cared for. Human life has always included infection, and sepsis in contemporary surgery still poses a serious challenge to medical professionals worldwide.

A microbiota is formed when bacteria that have colonized the wound bed come together due to exposure to external bacteria from the skin defect. Wound microbiota leads to wound infection if the wound is not properly cared of, which raises patient and healthcare system costs and, as a result, raises mortality. Three things need to come together for an infection to develop: an infectious host, an infection source, and a transmission pathway. A deeper comprehension of the variables influencing the diversity and composition of wound microbiota may lead to novel and preventive wound infection strategies, such as modifying those factors to prevent the formation of an unfavourable wound microbiota or change it in a more positive way.

Ācarya Susruta enlightened the importance of *Vraṇaśodhana* while discussing about *Saptopakrama*[3] and *Ṣaṣṭī upakrama*[4] in the context of *Vraṇa chikitsa*. The word *Vraṇaśodhaka* [5] means making the wound free of doshas and yields wound healing. *Paṭolanimbapatra Arka* [6], a *Vraṇaśodhaka Arka* is mentioned in *Arkaprakāśa* where the fresh leaves of *Paṭola* [7] and *Nimba*[8] is being used for the preparation of *arka*. As per *Bhāvaprakāśa nighaṇṭu* both the drugs are having *krimighna, pittahara* properties and they are indicated in *Vraṇa. Prakriti vighātam* is one among Trividha cikitsa of *Krimi*.Thus by providing healthy environment wound healing can also be promoted. Surgical wounds of Fissure-in-Ano are at risk of infections, as it also gets contaminated during bowel evacuation. So there exist a need for creating sterile environment that prevent delayed wound healing. These properties are classically attributed to *Paṭolanimbapatra Arka* as it is explained under the *Vraṇaśodhaka Arka*[6]. Restraining factors favorable for wound infection and by maintaining beneficial microbiota, wound healing can also be prompted. Thus, an attempt was made to comprehend

the effect of *Paṭolanimbapatra Arka* on wound microbiota as well as its wound healing property in surgical wounds of Fissure-in-Ano.

2. Methodology

2.1 Study design

The study was a Pre and post-test clinical study. The study was approved by ethics committee and registered with India's clinical trial registry of India (CTRI/2022/07/04420). Following informed consent, each patient was evaluated for eligibility to participate in the study.

2.2.1 Inclusion Criteria

- Age group - 20 to 60 years irrespective of gender and socio-economic status with Surgical wounds of Fissure in ano
- Surgical wounds on the anterior or posterior midline of the anal verge.
- Post-operative period, after 48hrs.
- Patient who has voluntarily agreed to participate in the study.

2.2.2 Exclusion Criteria

- Fissurectomy along with Fistula operated with *Kṣārasutra.*
- Patient with post operative complications like severe bleeding and pain.
- HBsAg and HIV positive patients
- Patient not willing to undergo trial.

2.3 Settings and location where the data was collected

OPD and IPD of Department of Śalyatantra, Amrita School of Āyurveda, fulfilling the eligibility criteria were recruited in the present study.

2.4 Intervention

Study medicine, *Paṭolanimbapatra Arka* is a classical poly-herbal formulation made of fresh leaves of *Paṭola (*Trichosanthes dioica-Collection no:95993) and *Nimba (*Azadirachta indica-Collection no:98789). The study drug was prepared as per the Arka Kalpana method. Fresh leaves of *Paṭola* and *Nimba* are collected and crushed. 1:10 part of drug and water was taken and kept overnight for soaking. Next day morning it was poured into *Arka yantra* and boiled. The vapours get condensed and collected in a receiver. This was stored in air tight container. The microbial load assessment of the prepared Arka was done at ACARA, Amrita School of Ayurveda and the result showed no microbial contamination.

Post-surgically (after 48 hrs. of surgery) local examination of participants with Surgical wounds of Fissure on the anterior or posterior midline of the anal verge were done. After sitz bath using

plain warm water, the surrounding area were cleansed using normal saline. The wound was cleaned using sterile cotton swab soaked in *Arka*. After proper cleaning, a gauze soaked in *Arka* was placed and sterile wound dressing was done once in a day. No adverse event was reported during the course of intervention.

2.5. Outcomes

2.5.1 Primary outcomes

Assessment of wound microbiota - Microbial load, Presence of Pathogenic organisms were assessed on 1st and 14th day by swab collected using Levine's technique before (BT_1) and 2hrs after application of *Arka* (AT_1).[9]

2.5.2 Secondary outcomes

The wound healing assessment was done on 1st,7th, 14th, 21st and 28th day using Bates-Jensen Wound Assessment Tool and the Percentage of healing assessment done using Barber Measuring Tool.

2.5 Sample size

20 participants fulfilling inclusion and exclusion criteria from the OPD and IPD of Department of Śalyatantra, Amrita School of Āyurveda were selected for the study.

2.6.1 Statistical analysis

It was done using Friedman Test to compare the parameters between the treatment stages and pair wise comparison was done using Wilcoxon signed rank test.

2.6.2 Demographic detail and baseline data

Here in this study out of 20 participants, majority of the them belonged to the fourth decade i.e., 45% of participants were in the group of 31-40 years; 55% of participants were female and 45% were male; 95% of the participants were taking mixed diet and hardly 5% were vegetarians and 45% belonged to *Vata-Pitha Prakṛti* and 35% belonged to *Vāta - kapha Prakṛti*.

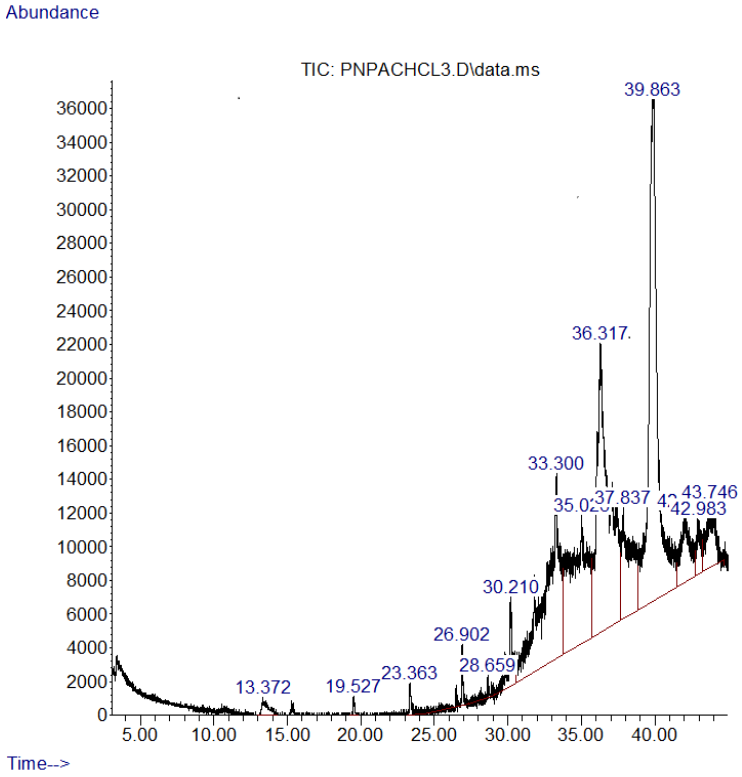
3. Results

3.1 Gas Chromatography-Mass Spectrometry Analysis of *Paṭolanimbapatra Arka*

Table No. 1: GC-MS Report of *Paṭolanimbapatra Arka*

SL.NO.	COMPOUND NAME	RETENTION TIME
1.	3 Nitrobenzotrifluoride	13.369
2.	Isoxazole	19.526
3.	1- Piperideine	23.366
4.	Behenic alcohol	26.902
5.	Cis-4-Ethoxy-b-methyl-b-nitrostyrene	28.658
6.	Hentetracontan-1-ol, 17-Pentatriacontene	30.209
7.	2-Octyldodecan-1-ol	33.299
8.	2-Ethylacridine	35.027
9.	Pentafluoro propionic acid	36.314
10.	4-hydroxy-3-nitrobenzotrifluoride	37.836
11.	9-O-Pivaloyl-N-acetylcolchinol	39.862
12.	Hexamethylcyclotriloxane	42.071
13.	Hexamethylyclotrisiloxane, 2-Ethylacridine	42.980
14.	2,4-Dimethylbenzo(H)quinoline	43.747

Graph No.1 Chromatogram of *Paṭolanimbapatra Arka*



Antimicrobial compounds[10,11,12]

Isoxazole, 1- Piperideine, Behenic alcohol, 2 Octyldodecan-1-ol, 4-hydroxy-3-nitrobenzotrifluoride, Hexamethylcyclotriloxane, 2-Ethylacridine, 2,4-Dimethylbenzo(H)quinoline, 9-O-Pivaloyl-N-acetylcolchinol, Pentafluoropropionic acid.

Anti- inflammatory compounds[13]:

3 Nitrobenzotrifluoride, Isoxazole, 1- Piperideine, 2-octyldodecan-1-ol, 17-Pentatriacontene.

Anti-oxidant compounds[14]:

Hentetracontan-1-ol, Hexamethylcyclotriloxane, 2-Ethylacridine

3.2 Results of Clinical Study

I. Assessment of Wound Microbiota by Surgical wound swab culture.

i. Microbial Load

Table No. 2: Assessment of Microbial Load between the treatment stages

Parameters	Time points	Mean	Std. Deviation	Mean Rank	Chi-Square	p value
MICROBIAL LOAD	BT_1	249.25	57.404	3.95	51	<0.001
	AT_1	166.4	56.8	2.65		
	BF_1	138.55	76.575	2.35		
	AF_1	93.7	60.357	1.05		

Friedman Test, *Significant at 0.05 level

A highly significant difference in the microbial load between the treatment stages with p value <0.001 (Graph no.2) was found.

ii. Pathogenic Organism

Table No.3: Assessment of Pathogenic Organism between the treatment stages

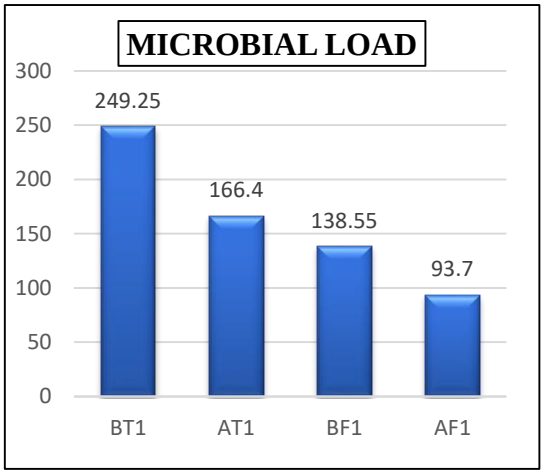
Pathogenic organism	BT1		AT1		BF1		AF1	
	n	%	N	%	n	%	n	%
No microbes	0	0	5	25	1	5	9	45
Beta Haemolytic Streptococci	2	10	4	20	0	0	0	0
E. Coli	11	55	4	20	11	55	7	35

Friedman Test	
$\chi 2$	p

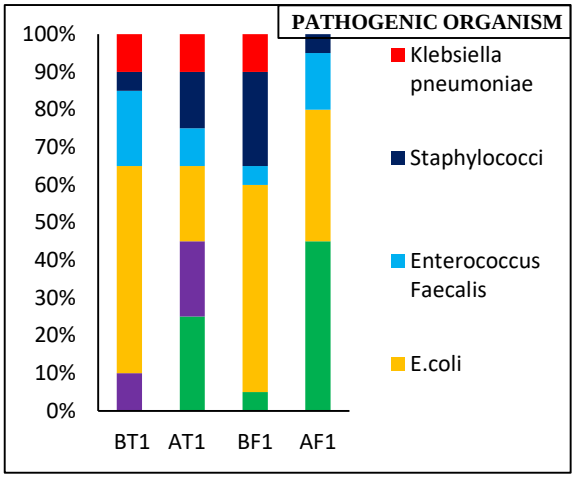
Enterococcus Faecalis	4	20	2	10	1	5	3	15
Staphylococci	1	5	3	15	5	25	1	5
Klebsiella pneumoniae	2	10	2	10	2	10	0	0
Total	**20**	**100**	**20**	**100**	**20**	**100**	**20**	**100**

11.98	0.007

Therefore, we can conclude that there is a significant difference in the presence of Pathogenic Organisms between the treatment stages with a p value < 0.05. (Graph no.2).



Graph 2: Representation of Microbial Load AT with respect to BT



Graph 3: Representation of Pathogenic organisms.

Microbial Load Assessment of Surgical wound of Fissure by Swab Culture

Results of 100µl (10-1diluted) sample spread over the [1]NA media and incubated for 24 hours at 37°C

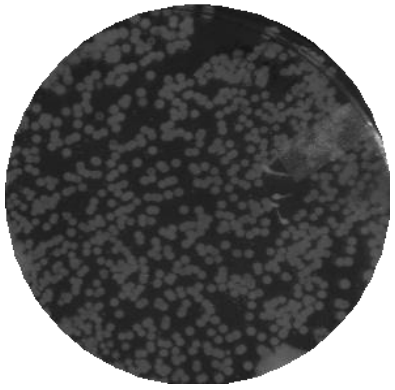


Fig 1: Distribution of Bacterial Colony Colony [2]BT_1

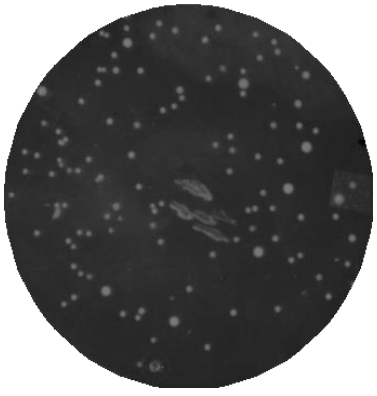


Fig 2: Distribution of Bacterial [3]AT_1

[1] NA media - Nutrient Agar Media (for bacteria)
[2] BT_1 – Swab before application of Arka on 1st day
[3] AT1-Swab after application of Arka on 1st day

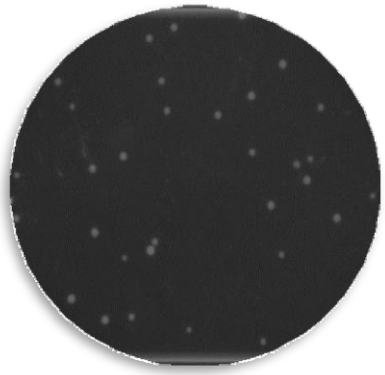


Fig 3: Distribution of Bacterial colony Bacterial colony[4]BF_1

Fig 4: Distribution of [5]AF_1

II. Wound Healing Assessment

i. By Bates-Jensen Wound Assessment Tool

Table No.4: Assessment of Wound Healing by Bates-Jensen Wound Assessment Tool

Parameters	Time points	Mean	Std. Deviation	Mean Rank	Chi-Square	p value
SIZE	BT	1.7	0.47	4.22	44.8	<0.001
	AT_1	1.35	0.489	3.35		
	AT_2	1	0	2.48		
	AT3	1	0	2.48		
	AT4	1	0	2.48		
DEPTH	BT	2.95	0.224	4.53	71.041	<0.001
	AT_1	2.75	0.444	4.15		
	AT_2	2.05	0.224	2.7		
	AT3	1.95	0.224	2.5		
	AT4	1.1	0.308	1.13		
EDGE	BT	2.75	0.55	4.6	67.928	<0.001
	AT_1	2.25	0.444	3.9		
	AT_2	1.9	0.308	3.18		
	AT3	1.3	0.47	1.88		
	AT4	1.05	0.224	1.45		

[4] BF1- Swab before application of Arka on 14th day
[55] AF1- Swab after application of Arka on 14th day

EXUDATE TYPE	BT	3.3	0.657	4.93	65.759	<0.001
	AT1	2.2	1.005	3.43		
	AT2	1.65	0.933	2.63		
	AT3	1.1	0.447	2.05		
	AT4	1	0	1.98		
EXUDATE AMOUNT	BT	2.95	0.224	4.75	68.621	<0.001
	AT1	2.35	0.489	3.65		
	AT2	2	0.324	2.98		
	AT3	1.75	0.444	2.48		
	AT4	1	0	1.15		
SKIN COLOUR	BT	2.15	0.366	3.93	52.618	<0.001
	AT1	2	0	3.58		
	AT2	1.9	0.308	3.33		
	AT3	1.7	0.47	2.83		
	AT4	1.1	0.308	1.35		
GRANULATION TISSUE	BT	4.55	0.51	4.93	78.021	V<0.001
	AT1	3.7	0.47	4		
	AT2	2.85	0.366	3.03		
	AT3	1.95	0.224	2		
	AT4	1.05	0.224	1.05		
EPITHELIALIZATION	BT	5	0	4.97	78.574	V<0.001
	AT1	4	0.324	4		
	AT2	2.95	0.224	3.03		
	AT3	1.95	0.224	1.73		
	AT4	1.5	0.513	1.27		

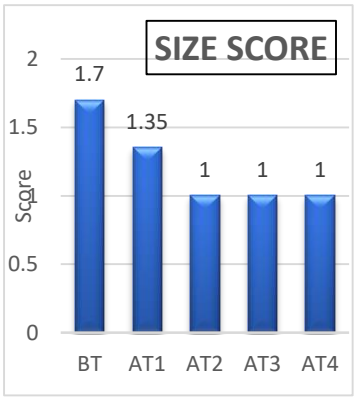
Friedman Test, *Significant at 0.05 level

The statistical analysis before and after treatment for the effects of therapy on various measures like Size, Depth, Edge, Exudate type, Exudate amount, Skin colour surrounding wound, Granulation tissue and Epithelisation using Friedman Test was found significant with a p value V<0.001.

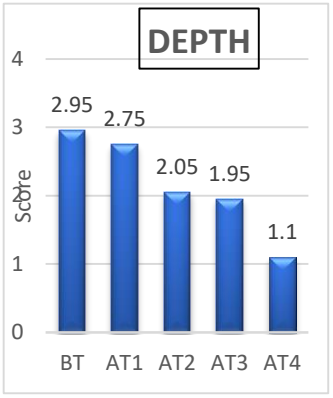
Parameters	**Time points**	**Mean**	**Std. Deviation**	**Mean Rank**	**Chi-Square**	**p value**
PERIPHERAL TISSUE EDEMA AND INDURATION	BT	1.1	0.308	3.2	8	0.092
	AT1	1	0	2.95		
	AT2	1	0	2.95		
	AT3	1	0	2.95		
	AT4	1	0	2.95		

Friedman Test, * Not Significant at 0.05 level

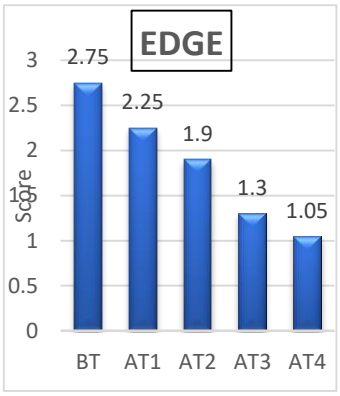
The parameters including Peripheral tissue oedema and Peripheral tissue induration was found not significant with a p value V>0. 05.



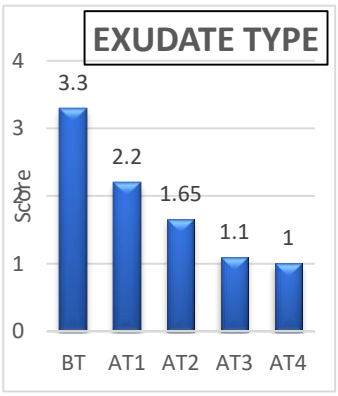
Graph 4: Representation of SIZE AT with respect to BT



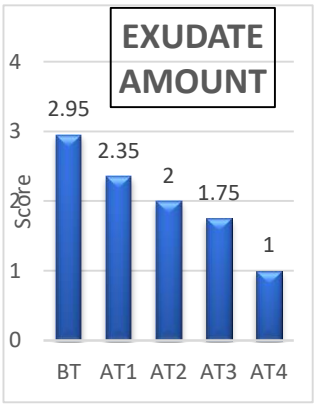
Graph 5: Representation of DEPTH AT with respect to BT



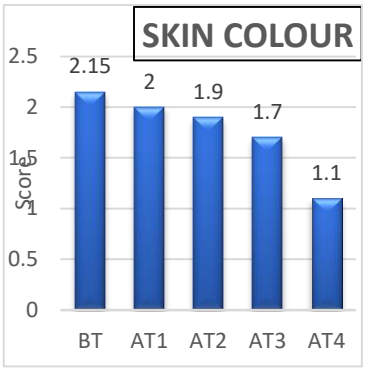
Graph 6: Representation of Edge AT with respect to BT



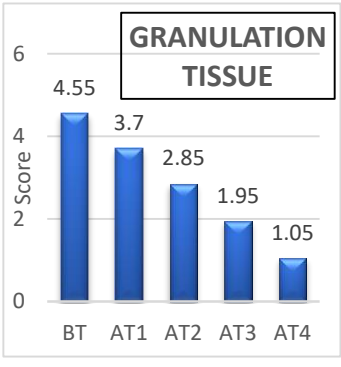
Graph 7: Representation of Exudate type AT with respect to BT



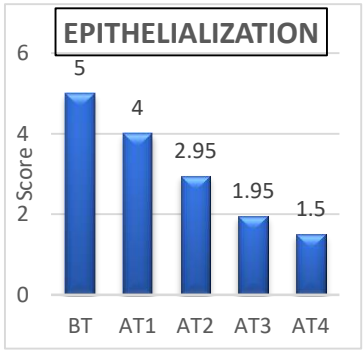
Graph 8: Representation of Exudate amount AT with respect to BT



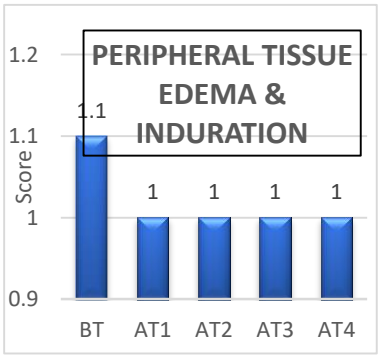
Graph 9: Representation of Skin colour AT with respect to BT



Graph 10: Representation of Granulation tissue AT with respect to BT



Graph 11: Representation of Epithelialization AT with respect to BT



Graph 12: Representation of Peripheral tissue induration AT with respect to BT

a. Volume

Table 5: Assessment of Volume between the treatment stages

Parameters	Time points	Mean	Std. Deviation	Mean Rank	Chi-Square	p value
VOLUME	BT	4.0	2.2	3.95	51	<0.001
	AT1	2.5	1.6	2.65		
	AT2	0.8	0.5	2.35		
	AT3	0.2	0.1	1.05		
	AT4	0.0	0.0	1.27		

Friedman Test, *Significant at 0.05 level

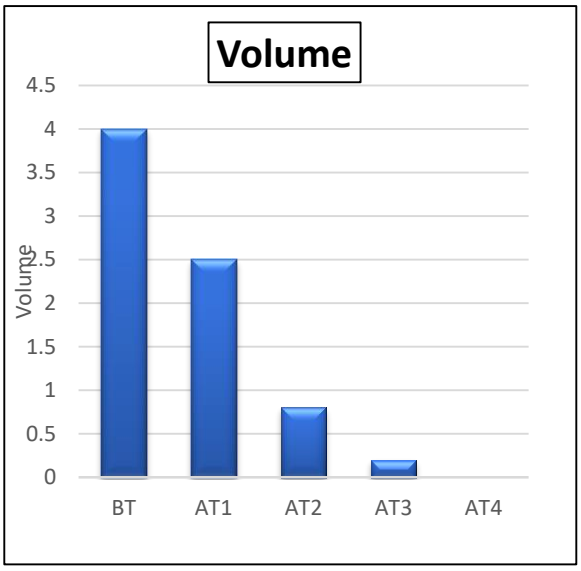
The Volume between the treatment stages was found highly significant with a p value < 0.001 **(**Graph 14).

b. Percentage of Healing

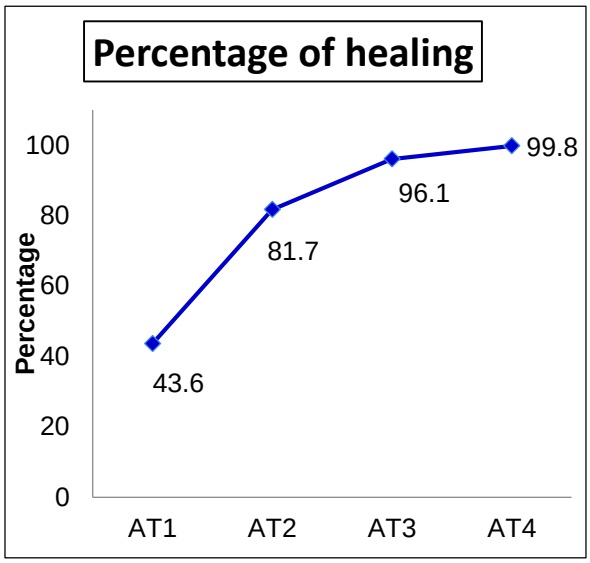
Table 6: Assessment of Percentage of Healing between the treatment stages

	N	Percentage of healing	
		Mean	Std. Deviation
AT1	20	43.6	17.3
AT2	20	81.7	7.1
AT3	20	96.1	2.7
AT4	20	99.8	0.5

Here the mean percentage of healing at AT1, AT2, AT3 and AT4 are 43.6, 81.7, 96.1and 99.8 respectively. Thus, there exists a significant improvement in the percentage of healing between the treatment stages (Graph 15).



Graph 14: Representation of VOLUME AT with respect to BT



Graph 15: Representation of PERCENTAGE OF HEALING AT with

Surgical wounds of Fissure-in-Ano Healing phases

Fig 5: Surgical wound before treatment BT

Fig 6: Surgical wound after treatment AT_4

4. Discussion

Surgical wounds of Fissure-in-Ano are susceptible to infection because they can become contaminated during bowel evacuation. In order to avoid delayed wound healing, a healthy environment must be created. The preservation of beneficial microbiota can also promote wound healing by limiting elements that are conducive to wound infection. *Paṭolanimbapatra* arka mentioned under Vraṇaśodhana Arka by Rāvaṇa confirms both antimicrobial and wound healing properties.

Discussion on *Paṭolanimbapatra arka*:

The individual drug of this formulation is fresh leaves of *Paṭola* and *Nimba*. According to *Ācarya Susruta*, both drugs fall into the category of drugs with *Vraṇa śodhana* and *Ropaṇa* properties. *Arka* formulations are more stable and has a longer shelf life, which was found advantageous for storage and administration in surgical wound of Fissure-in-ano.
On GCMS analysis of the *Arka* 14 compounds were identified that are having proven antibacterial, anti-inflammatory and antioxidant properties. Thus, the presence of these compounds suggests *Paṭolanimbapatra Arka* is having promising effect on wound microbiota and healing on surgical wound of Fissure in Ano.

Probable mode of action of *Paṭolanimbapatra Arka*

a. Effect on Wound microbiota:

Both the ingredients of the formulation viz. *Nimba* and *Paṭola* are of *Tikta, kaṭu* rasa respectively which attributes its *Krimighna karma*[15,16]. *Paṭola*, like most *Vraṇa-śodhana* plants, has these properties - *Kaṭu rasa*, *Uṣṇa vīrya,* and *Kaṭu vipāka*, can be used to cleanse contaminated wounds. As per *Āyurveda Nimba* is placed under *Vraṇaśodhana dravyas* which is having *Krimighna* properties.

The amorphous saponin and essential oil, present in Trichosanthes dioica must be responsible for its antimicrobial activity[17]. Azadirachta indica leaf extract includes a wide variety of chemical components, including phenols, tannins, protein, carbohydrates, alkaloids, and saponins, all of which support the plant's biological activity, including its antibacterial properties.[18]

GC-MS analysis report of arka revealed the presence of compounds having proven antimicrobial property that lowered the microbial load and created an atmosphere that is conducive to wound healing.

Before the treatment, five different micro-organisms were found among the participants, with E. coli being the most common. Trichosanthes dioica leaf extract is having proven antimicrobial properties against the bacteria Klebsiella pneumonia, Escherichia coli and

Staphylococcus aureus[19]. Azadirachta indica leaves is having proven antibacterial activity against the E. colistrains and S. aureus.[20]

b. Effect on Volume of the wound:

A decrease in volume of the wound was noted in every individual. It may be due to the fact that tissue binding action of *Nimba* due to its *Kaṣāya rasa* helps in wound closure and *Śīta Vīrya* for complete healing and remodelling phase in turn reduced the volume of wound[21]. The presence of Hentetracontan-1-ol and Hexamethylcyclotriloxane, both of which have proven antioxidative activity, was found during the analysis. Antioxidant compounds hasten wound healing by reducing oxidative stress and promoting faster skin cell regeneration.

c. Effect on Percentage of healing:

Each participant's percentage of healing showed a noticeable improvement. The possible reason for the improvement in the percentage of healing may be due to the *Śodhana* and *Ropaṇa karma* of *Nimba* and *Paṭola* i.e by cleansing the contaminated wound the healing got accelerated.

Peripheral Tissue oedema and Induration:

Post surgically inflammatory changes can be present in first two days and it starts to reduce by third day. As the participants in this study are included only after 48hrs of surgery, it was hardly observed in 2 participants so these parameters found statistically insignificant.

Undermining, Necrotic Tissue Type and Necrotic Tissue Amount:

The parameters in the Bates-Jensen wound assessment tool including Undermining, Necrotic tissue type and Necrotic tissue amount was not applicable in acute surgical wound. So, these parameters are not assessed in surgical wound of Fissure-in-Ano.

5. Conclusion

All participants showed statistically significant improvement in outcome measures like microbial load (<0.001), size (<0.001), depth (<0.001), edge (<0.001), exudate type (<0.001), exudate amount (<0.001), skin colour surrounding wound (<0.001), granulation tissue (<0.001), epithelialization (<0.001), volume (<0.001) and percentage of healing (99.8%).

Presence of Pathogenic organism- The presence of E. coli and Enterococcus faecalis was significantly reduced at AT_1(after 2hrs of application of arka). There was no significant reduction in the presence of Klebsiella pneumoniae, Staphylococcus aureus and Beta Haemolytic Streptococci at AT_1. At AF1, the presence of E. coli and Enterococcus faecalis decreased significantly, but Staphylococcus aureus did not decrease. Beta Haemolytic

Streptococci and Klebsiella pneumoniae was absent in all participants at AF_1. Among 20 participants no pathogenic organism was observed for 9 participants at AF_1.

Peripheral tissue oedema and Peripheral tissue induration was hardly observed in 2 participants before treatment and it got reduced at AT_1 itself.

The parameters in the Bates-Jensen wound assessment tool including Undermining, Necrotic tissue type and Necrotic tissue amount was not applicable in acute surgical wound.

Hence, *Paṭolanimbapatra Arka* impregnated gauze dressing was found effective in wound microbiota and healing in surgical wound of Fissure-in-Ano.

Surgical wound of Fissure-in-Ano which is at the risk of faecal contamination and infection can be effectively managed with *Paṭolanimbapatra Arka* impregnated gauze dressing.

Source of funding

None

Conflict of interest

No conflict of interest

References

1. Sriram Bhat M, SRB's Manual of Surgery, 6th edition, Jaypee Brothers medical publishers, New Delhi, Chapter 1, page 1
2. The Wound Microbiome: Modern Approaches to Examining the Role of Microorganisms in Impaired Chronic Wound Healing Ana M. Misic, Sue E. Gardner and Elizabeth Grice.
3. Prof. Sreekantha Murthy, Illustrated Susrutha Samhita, Vol1, Sutrasthana, Chapter 17 Amapakvaishaniya Adhyaya, sloka 18, Chaukhambha orientalia. Reprint 2016 edition), Page 127.
4. Prof. Sreekantha Murthy, Illustrated Susrutha Samhita, Vol 2, Chikitsa sthana, Chapter 1, Dvivraniya Chikitsa, sloka 8, Chaukhambha orientalia. Reprint 2019 edition), Page 6.
5. Prof. Sreekantha Murthy, Illustrated Susrutha Samhita, Vol 2, Chikitsa sthana, Chapter 1, Dvivraniya Chikitsa, sloka 9:7, Chaukhambha orientalia. Reprint 2019 edition), Page 7.
6. Dr. Indradeva Tripathi, Arkaprakasa of Lankapati Ravana. 1st ed. Varanasi: Chowkhamba Krishnadas Academy;1995; Shashtam shathakam: sloga number-18: 97.
7. Bhavaprakasha Nighandu, Sri Bhavamishra. Chaukhambha BharathiAcademy; Sakavarga:pg:no-676.
8. Bhavaprakasha Nighandu, Sri Bhavamishra. Chaukhambha Bharathi Academy; Guducyadi varga: pg:no- 328.
9. Tanner J, Dumville JC, Norman G, Fortnam M. Surgical hand antisepsis to reduce surgical site infection. Cochrane Database Syst Rev. 2016 Jan 22;2016(1):CD004288. doi: 10.1002/14651858.CD004288.pub3. PMID: 26799160; PMCID: PMC8647968.

10. Design of novel water-soluble isoxazole-based antimicrobial agents and evaluation of their cytotoxicity and acute toxicityAuthor links open overlay panelEvgeniy V. Kondrashov [a], Lyudmila A. Belovezhets [a], Nina S. Shatokhina [a], Alexandra N. Shilova [a], Yana A. Kostyro [a], Yulia A. Markova [b], Marina K. Borovskaya [b], Gennadii B. Borovskii [b]
11. In vitro Antibacterial and In silico Toxicity Properties of Phytocompounds from Ricinus communis Leaf Extract Sandile Nduduzo Mboyazi[1]*, Mduduzi Innocent Nqotheni[1], Tsolanku Sidney Maliehe[1], Jabulani Siyabonga Shandu[1]Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa 3886, SOUTH AFRICA
12. Epidihydropinidine, the main piperidine alkaloid compound of Norway spruce (Picea abies) shows promising antibacterial and anti-Candida activity Pia Fyhrquist a,*, Virpi Virjamo b, Eveliina Hiltunen b, Riitta Julkunen-Tiitto b a Division of Pharmaceutical Biosciences, Faculty of Pharmacy, P.O. Box 56, FIN-00014, University of Helsinki, Finland b Natural Product Research Laboratory, Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 111, Joensuu, Finland
13. Fathi M, Ghane M, Pishkar L . Phytochemical Composition, Antibacterial, and Antibiofilm Activity of *Malva sylvestris* Against Human Pathogenic Bacteria. Jundishapur J Nat Pharm Prod. 2022;17(1):e114164. https://doi.org/10.5812/jjnpp.114164.
14. Phytochemical profiling, antibacterial screening and antioxidant properties of the sacred tree (shorea robusta gaertn.) Of jharkhand raphael r. Marandi 1*, s. John britto 2 and prabhat k. Soreng 3 department of botany 1, st. Xavier's college, mahuadanr, nilamber-pitamber university, jharkhand 822119, india rapinat herbarium and centre for molecular systematics 2,st. Joseph's college (autonomous), bharathidasan university tiruchirappalli, tamil nadu - 620002, india department of botany 3, st. Xavier's college, ranchi university, jharkhand - 834001, india
15. Kunte am, navare ks, shastri hs, editors. *Ashtanga hridayam of vagbhata, sutra sthana. Ch. 10. Ver. 14. Reprint ed.* Varanasi: chaukhambha samskrita samsthana; 2017. P. 176. [google scholar]
16. Acharya yt, editor. *Charaka samhita of agnivesha, sutra sthana. Ch. 26. Ver. 42. Reprint ed.* Varanasi: chaukhamba sanskrit sansthan; 2016. P. 144. [google scholar]
17. A study of trichosanthes dioica (roxb): antibactericidial agent v. K. Saxena and r.k. dave
18. Phytochemical analysis and antibacterial properties of azadirachta indica (neem) leaves extract against e. Coli.
19. A novel antimicrobial agents trichosanthes dioica prashant kumar rai1, shikha mehta1, rajesh kumar gupta2 and geeta watal1*
20. Evaluation of phytochemical, antibacterial and free radical scavenging properties of azadirachta indica (neem) leaves.
21. kunte am, navare ks, shastri hs, editors. *Ashtanga hridayam of vagbhata, sutra sthana. Ch. 10. Ver. 7. Reprint ed.* Varanasi: chaukhambha samskrita samsthana; 2017. P. 175. [google scholar]