

**AN IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF KSHARASUTRA  
FORMULATION AGAINST PATHOGENS IN BHAGANDARA (FISTULA-IN-ANO)**<sup>1\*</sup>Dr. Samriti, <sup>2</sup>Dr. Pooja Sharma, <sup>3</sup>Deeksha Kumari, <sup>4</sup>Dr. Rijin Mohan, <sup>5</sup>Dr. Shijo Y. J.<sup>1</sup>MD Scholar, Department of Roganidana Evum Vikritivigyana, Shri Dhanwantry Ayurvedic College and Hospital 46 B, Sector 46 Chandigarh 160047.<sup>2</sup>MD Scholar, Department of Roganidana Evum Vikritivigyana, Shri Dhanwantry Ayurvedic College and Hospital 46 B, Sector 46 Chandigarh 160047.<sup>3</sup>Microbiologist, Department of Roganidana Evum Vikritivigyana, Shri Dhanwantry Ayurvedic College and Hospital 46 B, Sector 46 Chandigarh 160047.<sup>4</sup>Associate Professor, Department of Roganidana Evum Vikritivigyana, Shri Dhanwantry Ayurvedic College and Hospital 46 B, Sector 46 Chandigarh 160047.<sup>5</sup>Associate Professor, Department of Shalyatantra, Shri Dhanwantry Ayurvedic College and Hospital 46 B, Sector 46 Chandigarh 160047.**\*Corresponding Author: Dr. Samriti**MD Scholar, Department of Roganidana Evum Vikritivigyana, Shri Dhanwantry Ayurvedic College and Hospital 46 B, Sector 46 Chandigarh 160047. DOI: <https://doi.org/10.5281/zenodo.22160048>**How to cite this Article:** <sup>1</sup>Dr. Samriti\*, <sup>2</sup>Dr. Pooja Sharma, <sup>3</sup>Deeksha Kumari, <sup>4</sup>Dr Rijin Mohan, <sup>5</sup>Dr. Shijo Y. J. (2026). An In Vitro Evaluation Of Antimicrobial Activity Of Ksharasutra Formulation Against Pathogens In Bhagandara (Fistula-In-Ano).. World Journal of Pharmaceutical and Medical Research, 12(9), 150–156.

This work is licensed under Creative Commons Attribution 4.0 International license.

Article Received on 20/07/2026

Article Revised on 10/08/2026

Article Published on 01/09/2026

**ABSTRACT**

**Background:** *Bhagandara* (Fistula-in-ano) is a notoriously recurrent anorectal condition that Acharya Sushruta classified among the *Ashta Mahagada* due to its challenging management. Although *Ksharasutra* therapy remains a benchmark Ayurvedic intervention, limited scientific literature directly correlates its clinical success with microbiological assessments and Antimicrobial Sensitivity Testing (AST). **Aim:** This study evaluated the *in vitro* antimicrobial potency of two distinct *Ksharasutra* formulations against a pathogen isolated from an active *Bhagandara* tract. **Materials and Methods:** Purulent discharge was collected from a clinical fistulous tract and cultured on Nutrient and MacConkey Agar. The isolated pathogen was identified through Gram staining and standard biochemical tests (Indole, Catalase, Citrate). Two extracts - Extract A (*Guggulu*-based) and Extract B (*Snuhi*-based) - were tested at 100 mg/mL using the Agar Disc Diffusion method. Inhibition zones were compared against standard antibiotics (Amoxicillin-clavulanate, Meropenem) following CLSI guidelines. **Results:** The isolated pathogen was confirmed as *Klebsiella oxytoca*, a Gram-negative, lactose-fermenting bacillus. In the antimicrobial assay, Extract B (*Snuhi*-based) demonstrated superior activity with a 13 mm Zone of Inhibition (ZOI): outperforming Extract A (*Guggulu*-based, 9 mm ZOI) and notably exceeding the positive control, Amoxicillin-clavulanate (12 mm ZOI). **Conclusion:** These findings offer compelling, measurable evidence that *Ksharasutra* functions as more than a physical seton, it acts as an active, potent antimicrobial. The marked efficacy of the *Snuhi*-based formulation against *Klebsiella oxytoca* supports its traditional role in maintaining a *Shuddha Vrana*, driving simultaneous microbial clearance and tissue healing in complex anorectal fistulae.

**KEYWORDS:** *Bhagandara*, Fistula-in-ano, *Ksharasutra*, *Klebsiella oxytoca*, Antimicrobial Sensitivity Testing, Zone of Inhibition.

**INTRODUCTION**

The disease *Bhagandara* (Fistula-in-ano) is one of the most common anorectal disorders. Acharya Sushruta, listed eight especially serious diseases known as *Ashta Mahagada*. He included *Bhagandara* in this group, specifically highlighting it as a condition that is incredibly tough to heal and difficult to cure.<sup>[1]</sup> It is a

common reason for proctological surgery which can significantly affect patients' anorectal function and quality of life.<sup>[2]</sup> It begins as a painful boil or swelling (*pidika*) about two finger-breadths around the anal region (*guda marga*) and once the boil bursts, it develops into a fistula, termed *Bhagandara*.<sup>[3]</sup> Primarily five types of *Bhagandara* are described, though some texts mention

three additional types.<sup>[4]</sup> According to a study on the prevalence of anal fistula in India by the Indian Proctology Society, this disorder among the anorectal disorders affected roughly 17 to 20% of the population in different states, and 4% of new patients were reported to have it.<sup>[5]</sup> The intestinal microflora is a highly diverse ecosystem with over 400 bacterial species, dominated by anaerobes. While microbial count is low in the stomach and upper small intestine, it is high in the lower intestine, where bacteria exist both freely and attached to the mucosa, but normally do not invade the intestinal wall. This flora also includes organisms that can cause disease if they overgrow.<sup>[6]</sup> *Ksharasutra* therapy represents an Ayurvedic approach to managing *Bhagandara* (fistula-in-ano): utilizing a medicated thread to facilitate the simultaneous cutting, debridement, and healing of the fistulous tract. The thread is prepared with a coating of herbal alkalis and powders, chosen for their combined anti-inflammatory, antimicrobial, and cauterizing capabilities. While its clinical utility is well-recognized, contemporary research has yet to bridge the gap between traditional application and modern diagnostic precision. Specifically, there is a notable absence of studies that perform direct pus cultures from the fistula followed by Antimicrobial Sensitivity Testing (AST) to determine the Zone of Inhibition (ZOI) of the *Ksharasutra* against the specific isolated pathogens. By integrating these microbiological techniques, this study offers a novel,

evidence-based perspective on the antibacterial potency of the treatment. Correlating surgical intervention with documented microbial clearance provides a measurable scientific validation that is currently missing from the literature, effectively demonstrating the therapeutic impact of *Ksharasutra*.

## MATERIALS AND METHODS

### Sample collection

Sample was collected from patient presenting with symptoms of *Bhagandara* at the clinical facility. Under strict aseptic conditions, a sterile swab was used to collect the purulent discharge directly from the fistulous tract.

### Cultivation and Identification of bacterial isolates

The collected swab was immediately transferred to the laboratory for inoculation. The sample was streaked onto Nutrient Agar Media and incubated at 37°C for a duration of 24 to 48 hours to allow for microbial growth. The isolate was identified through a systematic approach involving colony morphology on selective and differential culture medium (MacConkey agar), Gram staining technique, and standard biochemical tests.

### Preparation of *Ksharasutra* extracts

Two experimental extracts were prepared for the study using *Ksharasutra* having components.

| Extract A |                 |                                |                      |
|-----------|-----------------|--------------------------------|----------------------|
|           | INGREDIENT      | SCIENTIFIC NAME                | FAMILY               |
| 1.        | Guggulu         | <i>Commiphora wightii</i>      | <i>Burseraceae</i>   |
| 2.        | Apamarga kshara | <i>Achyranthes aspera L.</i>   | <i>Amaranthaceae</i> |
| 3.        | Haridra         | <i>Curcuma longa L.</i>        | <i>Zingiberaceae</i> |
| Extract B |                 |                                |                      |
|           | INGREDIENT      | SCIENTIFIC NAME                | FAMILY               |
| 1.        | Snuhi ksheer    | <i>Euphorbia neriifolia L.</i> | <i>Euphorbiaceae</i> |
| 2.        | Apamarga kshara | <i>Achyranthes aspera L.</i>   | <i>Amaranthaceae</i> |
| 3.        | Haridra         | <i>Curcuma longa L.</i>        | <i>Zingiberaceae</i> |

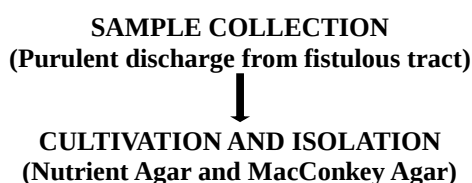
The coatings of the *Ksharasutra* are carefully scraped off using a sterile scalpel. A specific quantity (100mg) of the scraped material is dissolved in about (1mL) of sterile distilled water to prepare its extract.<sup>[7]</sup>

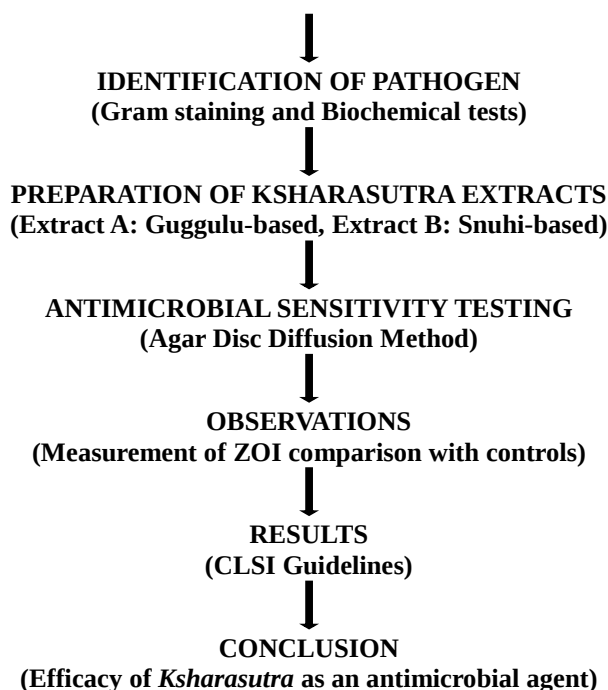
### Anti-bacterial activity of *Ksharasutra* by Agar disc diffusion method

To evaluate the antimicrobial efficacy, the Agar Disc Diffusion method was employed using clinical isolate from the fistulous tract. The bacterial inoculum was first standardized to adjust 0.5 McFarland standards, and then spread evenly across Mueller-Hinton Agar plate using

sterile cotton swabs to create a uniform lawn of growth. For the testing agents, the sterile discs of *Ksharasutra* Extract A (Guggulu-based) and Extract B (Snuhi-based) were saturated with 50 microliters of these extracts (100 mg/mL) and placed equidistantly on the dried agar surface alongside positive controls, Amoxicillin-clavulanate (20/10 µg)<sup>[8]</sup> and Meropenem (10 µg)<sup>[9]</sup> and a negative control, distilled water. Then the plate was incubated at 37°C for 18-24 hours. Significant susceptibility and resistance pattern was observed by following CLSI guidelines.<sup>[10]</sup>

## STUDY FLOW CHART





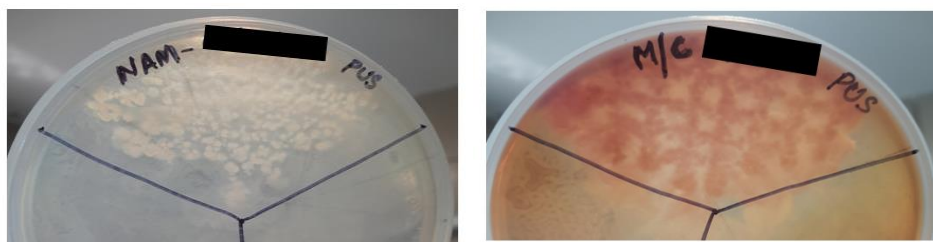
### OBSERVATIONS AND RESULTS

The microbiological analysis of the clinical sample successfully isolated an opportunistic pathogen from the purulent discharge of the fistulous tract. Initial cultivation on Nutrient Agar revealed distinct colony morphology, characterized by irregular, mucoid, grayish-white colonies. To further differentiate the organism, the isolate was cultured on MacConkey Agar - a selective and differential medium - where it produced large, pink, mucoid colonies; this clear evidence of lactose fermentation strongly pointed toward the *Klebsiella* genus. Microscopic examination via Gram staining confirmed the presence of Gram-negative, pink-colored, rod-shaped bacilli. To narrow down the specific species, a series of biochemical tests were performed. In the preliminary biochemical evaluation, the isolated bacterial strain was subjected to a series of diagnostic tests, demonstrating a positive reaction for catalase, citrate utilization, and nitrate reduction, while testing negative

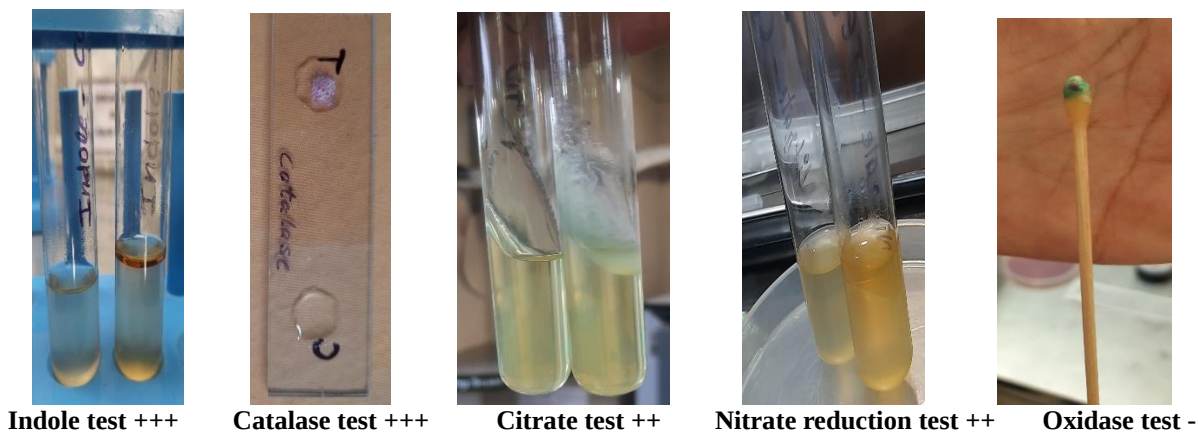
for oxidase. These initial results strongly indicated the presence of the genus *Klebsiella* (Table 1). To achieve definitive species-level identification and differentiate it from the closely related *Klebsiella pneumoniae*, an indole test was subsequently performed. The isolate tested positive for indole production, which is the key factor in distinguishing it from *Klebsiella pneumoniae*, the pathogen was identified as *Klebsiella oxytoca*. The antibacterial strength of the *Ksharasutra* extracts was then put to the test by measuring the Zone of Inhibition (ZOI) through the agar disc diffusion method (Table 2). Extract B (*Snuhi*-based) proved to be the more potent of the two, creating a 13 mm inhibitory zone, while Extract A (*Guggulu*-based) resulted in a 9 mm zone.<sup>[11]</sup> These results offer clear, measurable evidence of how these traditional medicated threads can actively suppress the growth of specific pathogens found in complex fistulous tracts.

**Table 1: Morphological and Biochemical Characteristics of the Isolate.**

| Feature                                                                                |                        | Observation                                        |
|----------------------------------------------------------------------------------------|------------------------|----------------------------------------------------|
| Colony Morphology<br>(Fig_1)                                                           | Nutrient Agar          | Colorless, irregular, mucoid                       |
|                                                                                        | MacConkey agar         | Lactose fermenting, pink, irregular, mucoid, large |
| Gram Staining                                                                          |                        | Pink-colored, Rod-shaped                           |
| Biochemical Tests<br>(Fig_2)                                                           | Indole test            | +++                                                |
|                                                                                        | Catalase test          | +++                                                |
|                                                                                        | Nitrate reduction test | ++                                                 |
|                                                                                        | Citrate test           | ++                                                 |
|                                                                                        | Oxidase test           | -                                                  |
| Note: '+++' shows strong positive, '++' shows moderate positive and '-' shows negative |                        |                                                    |



Fig\_1: Bacterial Growth on NAM and MacConkey Agar.



Indole test +++

Catalase test +++

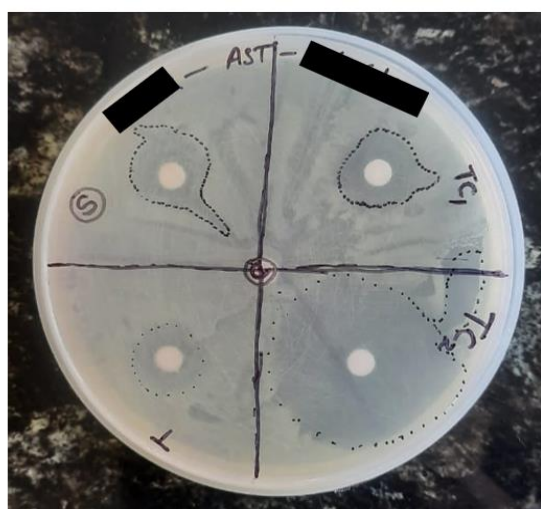
Citrate test ++

Nitrate reduction test ++

Oxidase test -

Fig\_2: Biochemical Tests.

| Table 2: Antimicrobial Sensitivity Testing Results (Fig_3) |                                |                                  |
|------------------------------------------------------------|--------------------------------|----------------------------------|
| Test Material                                              | Zone of Inhibition (ZOI) in mm | Interpretation (CLSI guidelines) |
| Ksharasutra Extract A (Guggulu-based T)                    | 9 mm                           | Resistant                        |
| Ksharasutra Extract B (Snuhi-based S)                      | 13 mm                          | Intermediate                     |
| Amoxicillin-Clavulanate (Positive Control TC1)             | 12 mm                          | Resistant                        |
| Meropenem (Positive Control TC2)                           | 22 mm                          | Intermediate                     |
| Distilled Water (Negative Control C)                       | 0 mm                           | -                                |



Fig\_3: Antimicrobial Susceptibility Testing (AST).

## DISCUSSION

The prevalence of anorectal conditions such as hemorrhoids, fissures and fistulas is on the rise, largely

driven by the complexities of modern living. Factors such as sedentary habits, poor dietary choices, extended periods of sitting, and mental health struggles like

anxiety and depression frequently disrupt metabolic health and digestion. This disruption often culminates in chronic constipation, which serves as the primary trigger for most anorectal ailments. Among these conditions, *Bhagandara* is particularly notorious for its stubborn nature and the significant discomfort it causes in daily life. Detailed descriptions of this pathology can be found throughout the classical Ayurvedic texts, including both the *Brihatrayi* and *Laghutrayi*. The development of *Ksharasutra* therapy has revolutionized the management of this disease. Through decades of rigorous clinical research and refinement, a standardized medicated thread was established, becoming the standard for treating *Bhagandara* and offering a highly effective alternative to conventional surgical methods.<sup>[12]</sup> The traditional method of fabricating *Apamarga Ksharasutra* requires 21 distinct, sequential coatings using a No. 20 surgical linen thread. In which ingredient plays a specific role in creating an active, slow-release local drug delivery matrix. Pure *Snuhi Ksheera* (latex of *Euphorbia neriifolia*): which forms a proteolytic enzyme base and primary binding adhesive. *Snuhi* latex layered with *Apamarga Kshara* (highly alkaline ash of *Achyranthes aspera*): providing a highly caustic and microbial-debriding layer. *Snuhi* latex layered with *Haridra Churna* (Turmeric powder, rich in curcumin): which seals the caustic thread, prevents ambient moisture absorption, and protects tissue.<sup>[13]</sup> This study provides a compelling and unique insight into the therapeutic mechanism of *Ksharasutra*. The primary strength lies in its innovative integration of an ancient Ayurvedic treatment with modern microbiological diagnostics. While the clinical efficacy of *Ksharasutra* has been observed for centuries,<sup>[14]</sup> this study provides scientific evidence that directly supports its antimicrobial claims. Anal fistula management is well studied, and recent work increasingly examines the antimicrobial potential of traditional therapies. While conventional treatments like fistulotomy and seton placement are well established, the medicated seton (*Ksharasutra*) is distinctive for its combined cutting and healing actions, offering an advantage over standard approaches.<sup>[15]</sup> Alkaline substances (*Kshara*) such as those from *Achyranthes aspera* (*Apamarga*) and curcumin from turmeric are known for broad-spectrum antibacterial and anti-inflammatory effects. Additional studies have shown significant antibacterial activity of *Achyranthes aspera* against various pathogens, supporting its anti-infective role.<sup>[16]</sup> A serine protease (*Neriifolin S*) from the latex of *Euphorbia neriifolia*. The enzyme exhibited strong proteolytic activity and broad substrate specificity, confirming that *Snuhi* latex contains biologically active proteases.<sup>[17]</sup> *Haridra* (*Curcuma longa*) provides sustained local anti-inflammatory activity through its principal bioactive constituent, curcumin. Curcumin suppresses key inflammatory mediators, including NF- $\kappa$ B and COX-2, thereby reducing the production of pro-inflammatory cytokines and prostaglandins. These actions help modulate excessive inflammation, promote wound healing, and may contribute to reduced local pain,

burning sensation, and tissue edema during *Ksharasutra* therapy.<sup>[18]</sup> Studies show they produce strong inhibition zones against pathogens like *Staphylococcus aureus* and *E. coli*, sometimes exceeding standard antibiotics. This offers an important clinical parallel to these laboratory findings. Chronic anal fistulas typically harbor enteric microbes like *E. coli*, *E. faecalis*, and *Bacteroides* spp.<sup>[19]</sup> The isolation of *Klebsiella oxytoca*, an opportunistic pathogen, aligns with known microbial diversity in fistulous tracts. Given rising antibiotic resistance, the effectiveness of a non-antibiotic therapy like *Ksharasutra* is particularly noteworthy. *Ksharasutra* demonstrated bactericidal action and reduced antibiotic resistance, highlighting its potential as an antibiotic-sparing strategy and its relevance in addressing the global resistance crisis.<sup>[20]</sup> *Snuhi Ksharasutra* keeps the tract clean, providing the *Shuddha Vrana Avastha* and only a *Shuddha Vrana* can heal properly. Due to *Vrana Ropana Karma* of the *Ksharasutra*, the healing process runs simultaneously.<sup>[21]</sup>

*Guggulu* is also indicated in *Vrana* (wound): *Apachi* (adenitis): *Pidika* (boils): *Granthi* (cyst) and *Shopha* (inflammation): which shows that *Guggulu* has antiseptic, anti-inflammatory and wound-healing properties and all these properties can increase the therapeutic effect of *Guggulu*-based *Ksharasutra*.<sup>[22]</sup> Evidence supports *Ksharasutra*'s antimicrobial activity, its alkaline components and herbal bioactive compounds create a hostile environment and exert direct cytotoxic effects on microbes. Modern medical sciences classify *Ksharasutra* as a sustained-release, chemical seton that causes controlled, sequential chemo-mechanical pressure necrosis. *Ksharasutra* therapy follows the principle of simultaneous cutting and healing. As the medicated thread gradually transects the fistulous tract (reported unit cutting time approximately 0.5–1.0 cm/week depending on tract characteristics): healing progresses behind the advancing thread through the normal wound-healing cascade involving angiogenesis, fibroblast proliferation, granulation tissue formation, and collagen deposition. Because only a limited portion of the sphincter complex is divided at any given time, progressive fibrosis and tissue repair help preserve sphincter integrity and maintain fecal continence.<sup>[23]</sup> That is how, the thread provides mechanical debridement, removing debris and preventing biofilm formation while chemical action promotes healing. So, we consider the *Ksharasutra* acts not only as a physical thread but as a potent bioactive antimicrobial agent. Integrating traditional therapies with modern diagnostic methods can strengthen scientific validation and support the exploration of non-antibiotic treatments for chronic infections like anal fistula, offering promise against rising antibiotic resistance.

## CONCLUSION

The study provides microbiological evidence supporting the antimicrobial potential of *Ksharasutra* formulations against *Klebsiella oxytoca*, an opportunistic pathogen

isolated from the fistulous tract of a patient with *Bhagandara*. Among the two formulations evaluated, the *Snuhi*-based *Ksharasutra* exhibited superior antimicrobial activity, suggesting that its therapeutic efficacy extends beyond the mechanical action of the medicated thread. This antimicrobial effect may be attributed to the synergistic action of *Apamarga Kshara*, which creates an alkaline environment unfavorable for bacterial survival, the proteolytic enzymes present in *Snuhi* latex, which facilitate debridement and microbial clearance and curcumin from *Haridra*, which contributes anti-inflammatory, antimicrobial, and wound-healing properties. Together, these bioactive components support simultaneous microbial suppression, tissue debridement, and wound repair, thereby providing a scientific basis for the traditional Ayurvedic concept of *Shuddha Vrana* and the simultaneous cutting and healing action of *Ksharasutra*. Considering the increasing burden of antimicrobial resistance, *Ksharasutra* may serve as a valuable adjunct or antibiotic-sparing strategy not only against *Klebsiella oxytoca* but also against other clinically relevant pathogens commonly associated with chronic fistulous infections, such as *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and anaerobic bacteria. These findings support the integration of traditional *Ksharasutra* therapy with modern microbiological approaches, thereby strengthening its scientific validation and expanding its potential clinical applicability in the management of chronic anorectal infections. While *Ksharasutra* is well documented in Ayurveda for managing *Bhagandara* (fistula-in-ano): this study highlights its microbiological relevance.

### LIMITATIONS

While this study provides valuable, novel evidence regarding the direct antimicrobial action of *Ksharasutra* formulations against a localized pathogen in *Bhagandara*, certain inherent limitations must be acknowledged. First, the identification of the clinical isolate was executed utilizing conventional phenotypic colony morphology, Gram staining, and core biochemical profiling. However, advanced molecular confirmation, such as polymerase chain reaction (PCR) amplification was not performed to genetically validate the *Klebsiella oxytoca* strain. Second, while the agar disc diffusion method demonstrated zone clearance, this preliminary in vitro design did not encompass quantitative minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), broth microdilution, or detailed mechanistic studies to map the exact down-regulation pathways of the herbal bioactives. Finally, because this evaluation was centered on a single, targeted clinical isolate from an active fistulous tract, the findings cannot be broadly generalized without caution. Future research profiles will focus on scaling this framework to include a significantly larger and more diverse repository of clinical isolates to comprehensively map susceptibility trends, determine precise MIC thresholds, and explore the broader molecular

mechanisms driving *Ksharasutra* efficacy against multi-drug resistant enteric pathogens.

**IEC APPROVAL:** Approved (IECSDACH/2026/013).

**FINANCIAL CONFLICT:** None.

### REFERENCES

1. Yadavji, T., 2012. Sushrut Samhita, Nibandh sangraha commentary. Chaukhambha Surbharti Prakashana, Varanasi, pp. 4–5.
2. Litta, F., Parello, A., Ferri, L., et al., 2021. Simple fistula-in-ano: is it all simple? A systematic review. *Tech. Coloproctol*, 25(4): 385–399. <https://doi.org/10.1007/s10151-020-02385-5>.
3. Sushruta, 2021. Sushruta Samhita, Ayurveda Tattva Sandipika Hindi Commentary, by Shastri A, Nidana Sthana, 4/3. Chaukhamba Sanskrita Samsthana, Varanasi.
4. Vagbhatta, 2021. Ashtanga Samgraha, Shashilekha vyakhya, uttar sthan 33/16-18. Chaukhamba Sanskrita Samsthana, Varanasi.
5. Sainio, P., 1984. Fistula-in-ano in a defined population. Incidence and epidemiological aspects. *Ann. Chir. Gynaecol*, 73(4): 219–224.
6. Gorbach, S.L., 1996. Microbiology of the Gastrointestinal Tract. In: Baron, S. (Ed.): *Medical Microbiology*, 4th edition. University of Texas Medical Branch at Galveston, Galveston (TX): Chapter 95. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK7670/>.
7. Kumara, P.A.A.J., Jayaratne, D.L., 2016. Antibacterial activity of ksharasutra (medicated setone): in the management of the fistula in ano. *Int. J. Res. Ayurveda Pharm*, 7(4).
8. Sathyavathy, K., Madhusudhan, B.K., 2021. Isolation, Identification, Speciation and Antibiotic Susceptibility Pattern of *Klebsiella* Species Among Various Clinical Samples at Tertiary Care Hospital. *J. Pharm. Res. Int*, 33(23A): 78–87. <https://doi.org/10.9734/jpri/2021/v33i23A31415>.
9. Ahmed Hasan, S., Mohammed Bakr, M., 2022. Bacteriological and Molecular Detection of *Klebsiella oxytoca* and its Resistance to Antibiotics among Clinical Specimens from Kirkuk, Iraq. *Arch. Razi Inst*, 77(5): 1521–1525. <https://doi.org/10.22092/ARI.2022.357753.2095>.
10. CLSI, 2026. Performance Standards for Antimicrobial Susceptibility Testing, 36th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, USA.
11. Tambekar, D.H., Dahikar, B.B., 2011. Antibacterial activity of some Indian Ayurvedic preparations against enteric bacterial pathogens. *J. Adv. Pharm. Technol. Res*, 2(1): 24–29. <https://doi.org/10.4103/2231-4040.79801>.
12. Mohite, M., et al., 2024. A Comparative Evaluation of Antimicrobial Effect of *Snuhi*-*Apamarg*-*Ksharasutra* and *Udumbara*-*Ksheer Sutra* in the Management of *Bhagandara* (Fistula-In-Ano). *Int. J.*

- Ayurvedic Med*, 14(4): 1015–1020.  
<https://doi.org/10.47552/ijam.v14i4.3965>.
13. Gupta, M.L., Gupta, S.K., Bhuyan, C., 2011. Comparative clinical evaluation of Kshara Sutra ligation and hemorrhoidectomy in Arsha (hemorrhoids). *Ayu*, 32(2): 225–229.  
<https://doi.org/10.4103/0974-8520.92591>.
  14. Dutta, G., Bain, J., Ray, A.K., Dey, S., Das, N., Das, B., 2015. Comparing Ksharasutra (Ayurvedic Seton) and open fistulotomy in the management of fistula-in-ano. *J. Nat. Sci. Biol. Med*, 6(2): 406–410.  
<https://doi.org/10.4103/0976-9668.160022>.
  15. Mohite, J.D., Gawai, R.S., Rohondia, O.S., Bapat, R.D., 1997. Ksharsootra (medicated seton) treatment for fistula-in-ano. *Indian J. Gastroenterol*, 16(3): 96–97.
  16. Kaur, M., Thakur, Y., Rana, R.C., 2005. Antimicrobial Properties of *Achyranthes aspera*. *Anc. Sci. Life*, 24(4): 168–173.
  17. Yadav, R.P., Patel, A.K., Jagannadham, M.V., 2012. Neriifolin S, a dimeric serine protease from *Euphorbia neriifolia* Linn.: Purification and biochemical characterisation. *Food Chem*, 132(3): 1296–1304.  
<https://doi.org/10.1016/j.foodchem.2011.11.107>.
  18. Akbik, D., Ghadiri, M., Chrzanowski, W., Rohanizadeh, R., 2014. Curcumin as a wound healing agent. *Life Sci*, 116(1): 1–7.  
<https://doi.org/10.1016/j.lfs.2014.08.016>.
  19. Jaiswal, P., Sharma, S., Pratap, A., Ansari, M., Shukla, V.K., Basu, S., Banerjee, T., 2021. Significant presence of biofilm-producing gut-derived bacteria in anal fistula of chronic duration. *Int. Wound J.*, 18(4): 519–524.  
<https://doi.org/10.1111/iwj.13551>.
  20. Radhakrishna Reddy, D., Shivalingappa, J.A., Ravi, R.C., 2022. Antimicrobial action of Tilanala Ksharasutra in the management of Bhagandara with special reference to fistula-in-ano. *Int. Ayurvedic Med. J.*, 5(5): 1118–1124.  
<https://doi.org/10.46607/iamj0410052022>.
  21. Nidhi Shree, K., Bibhuti, B., Panda, M., Arawatti, S., 2021. Clinical study of Snuhi Ksharasutra and its role in management of Bhagandara (Fistula in Ano). *J. Ayurveda Integr. Med. Sci*, 4: 17–23.  
<http://dx.doi.org/10.21760/jaaims.6.4.4>.
  22. Meena, R.K., Dudhamal, T., Gupta, S.K., Mahanta, V., 2018. Comparative clinical study of Guggulu-based Ksharasutra in Bhagandara (fistula-in-ano) with or without partial fistulectomy. *Ayu*, 39(1): 2–8.  
[https://doi.org/10.4103/ayu.AYU\\_19\\_15](https://doi.org/10.4103/ayu.AYU_19_15).
  23. Kirsner, R.S., Eaglstein, W.H., 1993. The wound healing process. *Dermatol. Clin*, 11(4): 629–640.