



## Research Article

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### ANTIBACTERIAL ACTIVITY OF *KSHARASUTRA* (MEDICATED SETONE), IN THE MANAGEMENT OF THE FISTULA IN ANO

A.A.J. P.Kumara <sup>1\*</sup>, D.L Jayaratne <sup>2</sup>

<sup>1</sup>Senior Lecturer, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Sri Lanka

<sup>2</sup>Senior Lecturer, Department of Microbiology University of Kelaniya, Sri Lanka

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**\*Corresponding author**

E-mail: mrsnhkumara@gmail.com

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#### ABSTRACT

Fistula in ano could be treated effectively with *Ksharasutra*, the surgical linen impregnated with special Ayurvedic medicine of alkaline in nature. There are different theories in existence claiming the mode of action of the thread in fistula in ano. Antibacterial activity of the *Ksharasutra* thread is one of it. Aim of this study is to identify the antibacterial activity of the *Ksharasutra*. Main objective is to determine the antibacterial activity by using Antibacterial Sensitive Test (ABST). The (ABST) test bacteria were *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa*, isolated from fistula specimens (Clinical cultures), and the standard strains. The *Ksharasutra* extract (100 mg/ml) was aseptically introduced into respective agar wells. Amoxicillin (100 mg/ml) was used as the positive control and the kept 50 µl distilled water was for the negative controls. Inhibition zones of  $\geq 7$  mm diameters around the well were regarded as significant susceptibility of the organisms to the extract. One way anova used as statistical method to analysis the data. Higher Zone of inhibition was observed in the *ksharasutra* thread extract for *Staphylococcus aureus*, 33.33±0. *Escherichia coli*, 30.60±0.33, *Streptococcus pyogenes* 24.005±0.57 and *Pseudomonas. aeruginosa*, 9.66±0.33. Standard antibiotic possess relatively low Zone of inhibition compare to the *ksharasutra* thread extract. Antibacterial activity of *Ksharasutra* due to the presence of *curcumin* and some of the inorganic compounds in *kshara* Cu, Zn Ca etc. So the *Ksharasutra* possess significant (P<0.05) Antibacterial activity in contrast to the standard antibiotic.

**Key words:** *Ksharasutra*, Fistula in ano, Antibacterial activity

#### INTRODUCTION

*Ksharasutra* treatment was considered as successful non-surgical procedure of treatment for the fistula in ano, to combat such critical anorectal problems. This comprehensive approach through Ayurveda has been extended with definite and a positive outcome. It is such a simple and safe remedy for anal fistula and it is becoming universally acceptable day by day. *Ksharasutra*. Treatment heals the fistulous tract with the integrity of sphincters. The existing data reveal that the negligible chances of recurrence after the treatment. The *Achyranthes. aspera Ksharasutra*. is well proven to be an effective treatment for fistula in ano and has been standardized by Central Council for Research in Ayurvedic Sciences (CCRAS), an apex research organization of the Government of India (GOI) in the field of Indian system of medicine<sup>1</sup> Sri Lanka Ayurveda Medical council and Department of Ayurveda have also recognized *Ksharasutra* treatment as a main parasurgical procedure for treatment of Fistula in ano.

According to the recent study conducted on the prevalence of anal fistula in India by Indian Proctology Society in a defined population of some states varied from 17% to 20%, while in a London hospital approximately 10% of all patients and 4% of new patients were reported to suffer from this disease among the anorectal disorders<sup>2</sup>.

*Ksharasutra* provides continue drainage of the abscess cavity or fistula track enhancing the drainage because *ksharasutra* act as a foreign body in the fistula track having caustic agents. The

effects include correction of the unhealthy tissues, enhancement of the healthy granulation tissue formation, enhancement of fibrolysis, separation of debris through the fistulous track, removal of debris and cleansing wound.

Bacteriology of anal fistulae has attracted with considerable interest over the past years. The major point of interest has been the finding that the presence of gut bacteria, as opposed to bacteria derived from skin, in the pus from anal abscesses that indicates an unsuspected anal fistula. Whether this is because tracks that connect with the ano rectum are more likely to be colonized by gut bacteria that virulent of unusual gut organisms are important as etiological factors in the genesis of anal fistula has never been investigated. These question were addressed by studying relationship between microorganism particularly bowel derived and skin derived.

During the *Ksharasutra* treatment no antibiotic or antimicrobial drug is administered to the patients. But the chronic granulating tract which usually occurred in ano rectal region frequently gets contaminant with faecal matter and harbor the microorganism. So far no research has been directed to assess antimicrobial activity of *Ksharasutra*, but it is compulsory to study this aspect of the treatment to justify it as a better alternative to the conventional surgical methods.

Objective of this research is to identify the antibacterial activity of the *Ksharasutra*

## MATERIALS AND METHODS

### Procedure of pus collection from fistula for the isolation of microorganisms

Anal fistulae are said to arise from cryptoglandular infection of the anal glands, which lie within the intersphincteric space. The microbiology of chronic anal fistulae has not been reported previously. Fifty consecutive anal fistulae were studied prospectively (22 intersphincteric fistulae, 12 trans-sphincteric fistulae, 12suprasphincteric fistulae, 3 extra sphincteric fistula, one superficial fistula and one ano vaginal fistula). There were 28 men and 22 women, with a median age of 42 (range 22–71) years.

Patients diagnosed with Clinical examinations and based on previous medical records were reviewed to identify patients with recurrent fistula in ano. Chronic perianal Crohn's fistulas, perianal and scrotal soft tissue necrosis, or pilonidal abscesses were excluded from the study. Age, duration of symptoms at admission, clinical symptoms, results of physical examination, risk factors, location of abscesses, antibiotics administered, and duration of treatment and hospitalization were recorded.

One hundred pus samples were collected from 50 patients. Prior to collection of pus smears, the perianal skin was cleaned using alcohol. Anorectal examination was carried out and anal canal and the lower rectum were carefully examined for internal openings by inspection and application pressure from outside to see if pus could be demonstrated. The pus smear was taken from the fistulous opening by sterile cotton swab to avoiding any contamination by local skin microflora and sent to the microbiology laboratory in sterile McCartney bottles and where pus cultured to isolate bacteria.

### Microorganisms isolated from pus samples and cultivation on agar media

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### Preparation of pure bacterial isolates

Morphologically different bacterial colonies were streaked on nutrient agar and were incubated at 37°C for 48 hrs. Isolated colonies were re-streaked several times on the same media. Purity of the culture was justified by microscopic observation via Grams stain. These isolates were then streaked on nutrient agar slants in stock bottles. These bacterial cultures were incubated at 37°C and stored at 4°C as stock culture.

### Identification of the bacterial isolates

Each of the isolates was examined for colony morphology, pigmentation, staining characteristics, shape and arrangements of cell, motility (hanging drop method), presence or absence of spores and finally biochemical tests. Identification of the bacterial isolates were carried out according to the standard methods given by the Bergey's manual of systematic bacteriology and identification of medical bacteria<sup>3</sup>

### Pour plate technique

The Muller Hinton Agar was allowed to cool until 45°C and 0.1ml of extract was transferred from each of the appropriate dilution to separate sterile labeled petri dishes, 15ml of the Muller Hinton Agar was added to each petri dish. The content were immediately mixed with the medium by a combination of to and fro shaking and circular movement.. The medium was allowed to solidify and the plates were incubated in an inverted position at room temperature approximately 18hours. The count was taken by using an electronic colony counter (maximum care was taken to minimize the time between preparation of dilution and the plating not exceed 15 minutes). Colony containing between 30 and 300 were selected for counting and every sample was tested with a control plate.

### Preparation of Ksharasutra extracts

The components of *Ksharasutra* were dissolved in normal saline and the concentration of 100mg/ml were made. For the preparation of *Ksharasutra*, of 4cm taken and dissolved in 1 ml Normal Saline. Extractions were collected in brown screw capped bottles and mixed at 200 rpm on 30 minutes vortex mixers. Then the shaken mixtures were filtered through Whatman No.01 filter paper and filtrate were collected for further use.

### Collection of test organism and preparation of stock culture

The test bacteria *Staphylococcus aureus*, *Streptococcus pyogen*, *Escherichia coli*, *Pseudomonas aeruginosa* were isolated from fistula in ano specimens. The standard strains (American Type Culture Collection (ATCC), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogen* (ATCC 19615), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and all other clinical isolates were obtained from stored samples at Department of microbiology, Faculty of medicine, University of Colombo. Above mentioned four types of clinical isolates were also tested in parallel to the type cultures. The stock culture of each isolates was prepared in taking two nutrient agar slants. One set slant was kept as stock culture and another as working set.

### Anti-bacterial activity of Ksharasutra by Agar well diffusion method

Antibacterial activity tests measure the ability of an antibacterial agent to inhibit bacterial growth in vitro .Bacterial broth culture

was prepared to a density of  $10^8$  cells  $ml^{-1}$  according to 0.5 McFarland standards. The aliquot was spread evenly onto Muller Hinton agar by sterile cotton swab. Then, the plated medium was allowed to dry at room temperature for 30 minutes on each plate, equidistant wells were made with a 6 mm diameter sterilized, cork borer, 2 mm from the edge of the plate.

Fifty micro liter of each *ksharasutra* extract (100 mg/mL) was aseptically introduced into a respective agar well. Amoxicillin (100 mg/mL) were used as positive controls and the distilled

water was included as negative controls. This was followed by allowing the agar plate on the bench for 40 minutes pre-diffusion followed by incubation at 37 °C for 24-48 h. The formation of clear inhibition zone of  $\geq 7$  mm diameters around the wells were regarded as significant susceptibility of the organisms to the extract. The experiment was performed in triplicate. Experiments that gave contradicting results were done for the third time for an easy decision.

**OBSERVATIONS AND RESULTS**

**Table 1: Bacteria isolated and categorized base on skin derived organisms and bowel derived organisms**

Skin derived organisms	No	%	Bowel derived organisms	No	%
<i>Staphylococcus aureus</i>	61	22%	<i>Enterococcus faecalis</i>	49	17%
<i>Corynebacterium spp</i>	04	1%	<i>Escherichia coli</i>	78	28%
			<i>Pseudomonas aeruginosa</i>	15	5%
			<i>Streptococcus pyogenes</i>	06	2%
			<i>Streptococcus agalactiae</i>	08	3%
Total	65	23%	Total	198	70%

In this study majority of fistula belong to the bowel derived organism accounting 70% while 23% were skin derived organisms. *Staphylococcus aureus* predominant among the skin derived organisms, *Escherichia coli* was the predominant bowel

derived organisms accounting 78%, *Enterococcus faecalis* and *Bacteriodes* represent 17% and 15% respectively. Bowel derived organisms were clinically important for fistula in ano is concern during the study. (Table 1).

**Statistical analysis**

**One way Anova was used as a statistical method to analysis of Zone inhibition**

**Table 2: Zone of inhibition for type cultures of Gram- negative and Gram- positive organisms for *Acyranthes aspera ksharasutra***

Bacteria	Mean±SE for <i>Ksharasutra</i>	Mean±SE for amoxicillin	t- value	Probability value
<i>Staphylococcus aureus</i> (ATCC 25923)	33.33±0.33	30.667±0.31	9.9	P<0.05 SIG
<i>Escherichia coli</i> (ATCC 25922)	31.33±0.33	20.80±0.30	16.26	P<0.05 SIG
<i>Streptococcus pyogen</i> ATCC (19615)	24.30±0.33	21.66±0.30	4.2	P<0.05 SIG
<i>Pseudomonas aeruginosa</i> ATCC (27853)	10.66±1.03	6±0		

*Ksharasutra* thread, the final product consist with *kshara*, latex and *curcuma longa*. The antibacterial effect of *Ksharasutra* was tested using amoxicillin as the standard antibiotic against ATCC type cultures of the Gram positive and Gram negative bacterial strains.

It was observed the Zone of inhibition of *ksharasutra* for all the tested bacteria including *Pseudomonas aeruginosa*. But standard antibiotic did not show any antibacterial activity against *Pseudomonas aeruginosa*. The highest Zone of inhibition for

ATCC cultures was observed in *ksharasutra* thread extract for *Staphylococcus aureus* 33.33±0.33. However *Escherichia coli*, 31.33±0.33, *Streptococcus pyogen* 24.30±1.38 and *Pseudomonas aeruginosa*, 10.66±0.5. Standard antibiotic possess relatively low Zone of inhibition compare to the *ksharasutra* thread extract; *Staphylococcus aureus* 30.667±0.3, *Escherichia coli* 20.8±0.3, *Streptococcus pyogen* 21.66±0.3. *Ksharasutra* thread extract was observed significant difference in Zone of inhibition in compare to the standard antibiotic. This difference was statistically significant. (Table 2).

**Table 3: Zone of inhibition for Gram negative and Gram positive organism *Achyranthes. aspera ksharasutra* for clinical culture**

Bacteria Clinical isolates	Mean±SE for <i>Ksharasutra</i>	Mean±SE for Amoxicillin	t- value	Probability value
<i>Staphylococcus aureus</i>	33.00±0.37	26.66±0.66	10.58	P<0.05 SIG
<i>Escherichia coli</i>	30.60±0.33	17.30±0.33	28.3	P<0.05 SIG
<i>Streptococcus pyogen</i>	24.00±0.57	17.60±0.33	9.5	P<0.05 SIG
<i>Pseudomonas aeruginosa</i>	9.66±0.33	6±0		

Antibacterial activity of *Ksharasutra* thread, using amoxicillin as the standard antibiotic for clinical cultures of Gram positive and Gram negative bacteria are shown in Table 3. It was observed that Zone of inhibition of *Ksharasutra* for all the tested bacteria include *Pseudomonas aeruginosa*, but the standard antibiotic didn't show antibacterial activity against clinical isolates. Higher Zone of inhibition was observed in the

*ksharasutra* thread extract for *Staphylococcus aureus*, 33.33±0. But *Escherichia coli*, 30.60±0.33, *Streptococcus pyogen* 24.00±0.57 and *P. aeruginosa*, 9.66±0.33 showed low Zone of inhibition. Standard antibiotic possess significantly low Zone of inhibition compare to the *ksharasutra*; *Staphylococcus aureus* 26.66±0.66 *Escherichia coli*, 17.3±0.33, *Streptococcus pyogen* 17.6±0.33 and no inhibition for *Pseudomonas aeruginosa*.

Accordingly the *Ksharasutra* thread extract showed high antibacterial activity compare to the standard antibiotic. This difference was statistically significant. (Table 3).

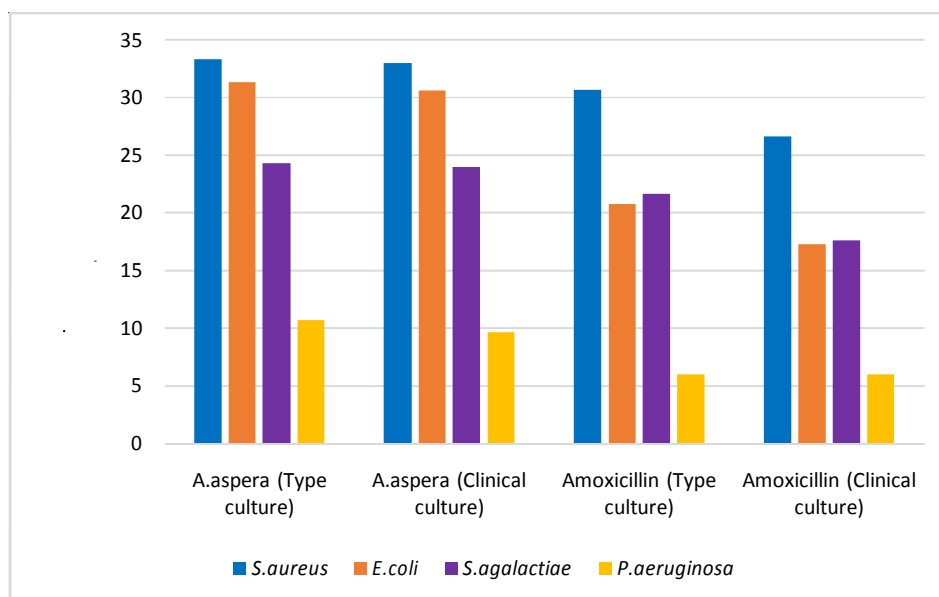


Figure 1: Zone of inhibition of type cultures and clinical cultures produced by, *Achyranthes aspera* Ksharasutra vs amoxicillin

It was observed that there were differences in Zone of inhibition for type cultures and clinical cultures against, amoxicillin. The higher Zone of inhibition for the tested bacteria ATCC type cultures showed as follows *Staphylococcus aureus*, 30.66±0.33, *Escherichia coli*, 23.66±0.33 and *Streptococcus pyogen*, 17.66±0.33. But amoxicillin had shown the lower Zone of

inhibition for the respective clinical cultures of *Staphylococcus aureus*; 26.66±0.66, *Escherichia coli* 17.33±0.33 and *Streptococcus pyogen* 17±0.33. So there were significant difference observed in comparison of Zone of inhibition for type cultures and clinical cultures against amoxicillin. This difference was statistically significant (Table 4, Figure 1).

Table 4: Differences of Zone of inhibition between the type cultures and clinical cultures for amoxicillin

Type of bacteria	Mean±SE for Type culture	Mean±SE for Clinical culture	t- value	Probability value
<i>Staphylococcus aureus</i>	30.66±0.33	26.66±0.66	5.37	P<0.05 <sup>SIG</sup>
<i>Escherichia coli</i>	20.08±0.33	17.33±0.33	13.4	P<0.05 <sup>SIG</sup>
<i>Streptococcus pyogen</i>	21.66±0.33	17±0.33	5.4	P<0.05 <sup>SIG</sup>

Table 5 Differences of Zone of inhibition between type cultures and clinical cultures for standard *ksharasutra* (*Achyranthes.aspera*)

Type of bacteria	Mean±SE for Type culture	Mean±SE for Clinical culture	t- value	Probability value
<i>Staphylococcus aureus</i>	33.33±0.33	33±0.57	1	P>0.05 <sup>NS</sup>
<i>Escherichia coli</i>	31.33±0.33	30.66±0.3	1.4	P>0.05 <sup>NS</sup>
<i>Streptococcus pyogen</i>	10.66±0.33	9.66±0.33	2.12	P>0.05 <sup>NS</sup>

It was observed that there were no differences in Zone of inhibition for type cultures and for clinical cultures against *Achyranthes aspera ksharasutra*, The High Zone of inhibition for the tested bacteria ATCC type cultures are as follows; *S. aureus*, 33.33±0.33 *Escherichia coli* 31.33±0.33 *Streptococcus pyogen*, 24.33±0.33 and *Pseudomonas aeruginosa* 10.66±0.33. But *ksharasutra*, had not showed significant difference in Zone of inhibition for the respective clinical cultures of *Staphylococcus aureus*; 33±0.57, *Escherichia coli*, 30.66±0.3 and *Streptococcus pyogen* 24±0.57 and *Pseudomonas aeruginosa* 9.66±0.33. So there were no significant difference was observed in comparison to type cultures and to clinical cultures. This difference was statistically not significant. (Table 5).

## DISCUSSION

Anal fistulae are said to arise from crypto glandular infection of the anal glands, which laying within the intersphincteric space.

This study was focused to identify the type and virulence of the common microorganisms responsible for the development of anal fistula. The microorganisms of chronic anal fistulae have not been reported previously. For the first time in this study the incidence of bacterial infection in fistula in ano and the origin of the predominant bacteria present in ano rectal fistula were investigated using 100 pus samples obtained from 50 patients. Accordingly 282 isolate were detected from 28 men and 22 women patients.

It was decided that the identification of all species of bacteria isolated from these specimens would be wasteful of time and effort. Therefore, the intention of this study was to divide the isolates into either skin-derived or bowel-derived organism. Accordingly Isolates of *Staphylococcus aureus*, and *Corynebacterium* spp. were identified as skin derived organisms and Isolates of *Enterococcus faecalis*, *Streptococcus pyogen*,

*Escherichia coli*, *Bacteriodes* spp were considered as gastro intestinal tract derived organisms.

The results of this study reasonably suggest that Enterobacteriaceae are the most common causative pathogens of peri-anal infection, and not *Streptococci* or *Staphylococci*, which are the most common causatives of other sites of skin and soft tissues infections. *Staphylococcus aureus* account for 21 percent of the organisms isolated from pus in chronic anorectal fistula. It was reported that *Staphylococcus aureus* forms only a very small proportion of the fecal flora<sup>4</sup> And the possibility of anal gland infection by *Staphylococci* is therefore low. If all ano-rectal fistulas are originate due to intersphincteric sepsis, a fistula should be found irrespective of the bacterial infection but no previous study has related bacterial infection to the occurrence of a fistula. In this study the pus from 23% were skin-derived in contrast to the isolates of 70% were bowel-derived. So this study suggests that the skin derived organisms, May less responsible for anal gland infection which leads to develop fistula in ano. (Table 1).The study also suggested that the bacterial colonization by variety of bowel derived organisms may occur in chronic anal fistulae were *E. coli* and *Bacteriodes* spp that are leading to pathogenesis of anal fistulae.

The highest Zone of inhibition for the Gram positive bacteria was *Staphylococcus aureus* while for Gram negative bacteria was *Escherichia Coli* against *ksharasutra*. The most resistance bacteria for *ksharasutra* was *Pseudomonas aruginosa*. Showed Zone of inhibition for the type culture 10.66±1.03 and for the clinical culture 9.66±0.33.

However *Achyranthes aspera ksharasutra*, had not statistically significant difference in Zone of inhibition for the clinical cultures or type cultures at P>0.05. Accordingly these bacterial cultures are equally sensitive to *Achyranthes aspera ksharasutra* (Table 5).

So this study clearly indicate that standard *Achyranthes aspera ksharasutra* possess the highest significant antibacterial activity proving its clinical efficacy. This antimicrobial activity of *ksharasutra* base on its individual components; of *Kshara*, *Curcuma longa* powder and binding agents, latex *Euphorbia antiqurum*. The antibacterial activity of *Curcuma longa* base on its organic compounds curcumin. Curcumin has also been shown to possess *in vitro* anti-microbial potential against wide range of microorganisms including fungi<sup>5</sup> as well as several Gram-positive and Gram-negative bacteria<sup>6</sup> It was recorded that the antibacterial activity of curcumin against *Bacillus subtilis* occurs through the inhibition of bacterial cell proliferation by blocking the assembly dynamics of Fts Z in the Z ring<sup>7</sup>. In the case of *P. aeruginosa* infection, curcumin was shown to have anti-infective activity through affecting the bacterial virulence, quorum sensing and biofilm initiation<sup>8</sup> Curcumin is involved in disordering the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) membranes<sup>9</sup>.

*Euphorbia antiqurum* latex possess mild antibacterial activity for *Staphylococcus aureus* and *Escherichia. Coli*<sup>10</sup> In *ksharasutra* preparation *Euphorbia antiqurum* latex is used as a binding agent, traditional extracts of the plant are used in sore and wound healing as ear drop for boils in the ear and treatment of boils. Higher Zone of inhibition exhibited by the extract against *Staphylococcus aureus* justified their use by traditional medicinal practitioners in the treatment of boils, sores and wounds<sup>11</sup>. *Ksharasutra* therapy of fistula seems to make good use of chemical cauterization with a medicated thread. As binding agent of the thread *Euphorbia antiqurum* latex is natural mild herbal base which performs the uniform and

smooth cutting of normal tissue and the abnormal granulation as well and thereby reduces the depth of fistula with no or least recurrences<sup>12</sup>

The diverse composition of the latex, which includes toxic compounds as well as other potentially bioactive molecules such as diterpenes and triterpenes could be responsible for the antibacterial and anti-fungal activity<sup>13,14</sup>

Clinical efficacy of *Apamaraga ksharasutra* has been proved in this study and our findings tally with previous study<sup>15</sup>

## CONCLUSION

*Ksharasutra* treatment is a reliable and non-ambulatory cure in the management of fistula in ano without complication and recurrences. So the *Ksharasutra* ligation treatment offers a good ray of hope. Its gradual but sustained chemical action removed the debris from the site of fistula and it also helps in formation of healthy granulation tissue thereby inducing a long healing pattern in depth of the tissue. *Ksharasutra* also dissolves the tough fibrous tissue and ultimately drains creating a healthy surrounding for healing and its antibacterial activity helping to control the infection of fistula in ano as well as reduce the bacterial density which leads to speed recovery of the disease.

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