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Original Article

# Chemical Analysis of *Ksharasutra* (Medicated Setone) In the Management of Fistula in Ano

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ABSTRACT

The parasurgical approach by application of ksharasutra for the treatment of Ano-rectal diseases was first described in the Sushrut samhita. Fistula in ano could be treated effectively with Ksharasutra, the surgical linen impregnated with special Ayurvedic medicine of alkaline in natureThe usefulness of the method still very relevant and is practiced by the Ayurvedic practitioners since long. In this article a scientific approach has been undertaken to evaluate the natural compounds present in the individual Ayurvedic plant ingredients used for the preparation of the Medicated thread (ksharasutra).Basic bio chemical tests, TLC and GC MS tests were carried out to identify the chemical compounds in the thread. A good number of chemical present in the ksharasutra like Euphol, Neriifolin which are possess activities like anti inflammatory, antibacterial & antifungal, Water soluble ash of Apamarga (whole plant) contains Betaine. Pharmacologically rhizome of haridra possesses anti-inflammatory, antibacterial, antifungal, anti infective, analgesic and anti puritic effect is due to the presence of active constituents Curcumin , Betaine. .6-Dodecene, 3-Hexadecene

**Keywords:** ksharasutra, Apamarga kshara, Haridra.

## 1. INTRODUCTION

The *Ksharasutra* procedure is an effective non-surgical treatment for the fistula in ano. The operative technique are uncertain and the recurrence rate is (11to 44) % [1, 2]. Apart from this, the operative management has some adverse effect like incontinence of feces, loss of gluteal cushion and postoperative stenosis. The treatment of Fistula in ano by *Ksharasutra* is very simple, easy and safe with least recurrences. It is one

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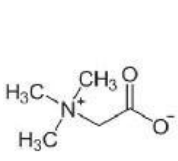
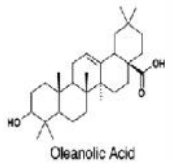
among popular Ayurvedic treatment modality in the branch of *Salyatantra.Ksharasutra* was first mentioned by the "Father of Surgery" *Sushruta* in his text named *Sushrut-samhita* for the treatment of *Nadivrana* [3] .

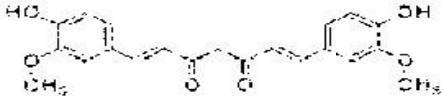
Application of *ksharasutra* in healing the fistulous track is an unique & effective parasurgical therapy. The technique served to achieve what he termed as chemical fistulectomy in contrast to the physical fistulectomy achieved by surgery. Among the three ingredients of the thread, the latex of*Euphorbia Antiqurum*is well known in Ayurveda for its wound healing property, whereas *Achyranthes aspera kshara* is considered necessary for ensuring the alkaline medium without which the thread is not at all effective. *Curcuma longa* powder is used as the last coating on the thread to aid in minimizing the severe local reaction due to the caustic action of the other two herbal ingredients. And also for its known anti-inflammatory and antibacterial effect.[4] The *Ksharasutra* treatment is an unique therapy for the management of fistula in ano. It is no doubt a treatment of relatively longer duration but the patient is ambulatory throughout the procedure and need not refrain from his daily routine work. Presumably the caustic action of the thread destroys the notorious cryptic gland completely which is supposed to be the primary seat of the origin of fistula [5]. *Apamarga kshara* (*Achyramthus aspera*) whole plant is to be collected and cut into pieces . After drying the plant is to be shade, it should be burnt in light fire. Ash is collected and is dissolved in six times of water. The solution, so formed, is filtered with the help of percolator. Residual ash is again dissolved in four times of water and the same procedure is repeated at least twice in order to take away all the alkaline material from the ash. Ultimately, the ash remains as a neutral residue which should be thrown. The fluid is filtered21 times (Su.Su.11/11) and finally, the *kshara* is obtained by evaporating the filtered solution. Latex (*Euphorbia Antiqurum*) is collected by giving perpendicular incision the stem of *Euphorbia Antiqurum* plant.. *Curcuma longa* dry rhizomes of '*Haridra'* plant are cut into pieces and make them powder.

The standard *ksharasutra* is prepared by repeated coatings of *snuhi ksheera* (Latex of*Euphorbia Antiqurum*), *Apamarga kshara* (ash of *Achyranthus aspera*) and *Haridra* powder over a surgical Barbour linen thread no. 20. This thread is spread out lengthwise in hangers each thread on the hanger is then smeared with latex with the help of gauze piece soaked in the latex. This wet hanger is transferred in *ksharasutra* cabinet. On the next day the dried threads are again smeared with *Euphorbia Antiqurum* latex, this process is repeated for 11 days. On the 12$^{th}$ day the thread is again smeared with *Euphorbia Antiqurum* latex and then in the wet condition, thread is spread over the *Apamarga kshara* powder. The thread is now allowed to dry in cabinet & the same procedure is repeated for seven times in seven days continuously. On 19$^{th}$ day the dried thread is smeared again with *Euphorbia Antiqurum* latex and in wet condition, *haridra* powder is to be coated over the thread & is repeated for three consecutive days. In this way, a thread has total 21 coatings of*Euphorbia Antiqurum* latex.






Betaine



**Betaine**

Activity:- Basic substance used as kshara, Antibacterial and Antifungal

## 2. METHODOLOGY

**Phytochemical analysis of *Ksharasutra***

The acetone extracts of *Ksharasutra* thread (brown color solution) were subjected to phyotochemical analysis to detect the presence of following biomolecules using the standard qualitative procedures as described by (Trease *et a*l., 1989). [6]

**Test for tannins:** To 0.5 ml. of extract solution, 1.00 ml. of distilled water and 1-2 drops of ferric chloride solution were added and observed for brownish green or a blue black coloration.

**Test for saponins:** The extracts of 5.00 ml. was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins.

**Test for flavonoids:** A few drops of 1% amonia solution was added to the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

**Test for cardiac glycosides:** The 1 ml of concentrated $H_2SO_4$ was taken in a test tube. 5.00 ml. of the extract was mixed with 2.00 ml. of glacial acidic acid containing 1 drop of $FeCl_3$. The above mixture was carefully added to I ml of concentrated $H_2SO_4$. Presence of cardiac glycosides was detected by the formation of a brown ring.

**Test for Alkaloids:** The extract 3.00 ml in a test tube mixed with 1.00 ml of 1% HCl and the mixture was treated with few drop of Mayer's reagent. A creamy white precipitate indicated the presence of alkaloids.

**Test for Glycosides:** The 10ml. of 50% $H_2SO_4$ was added to 1ml. of the extract in a boiling tube. The mixture was heated in boiling water for 5 min. 10.00 ml, of Fehling's solution (5.00 ml. of each solution A & B) was added and boiled. A brick red precipitate indicated presence of glycosides.

**Reducing Sugars:** 3.00 ml of test solution was added with a 2 ml of Fehling's reagent and 2.00 mlof water. Formation of reddish orange colorindicates the presence of reducing sugar.

**Sugars:** 3.00 ml of the test solution was addedwith very small quantity o fanthrone reagentand a few drops of concentrated $H_2SO_4$ and heated. Formation of green or purple color indicates the presence of sugars

**Separation of clinically important organic compound by TLC using different solvent System:**

*Curcuma longa* was a one of the main component used for the *ksharasutra* preparation, curcumin was the main active compound which responsible for the antibacterial activity of the thread. Betaine and Guggulesterone also prominent pharmacologicaly important compounds available in *Ksharasutra*Hence the TLC method was carried out to identify the curcumin and Betaine in *ksharasutra*.. *Ksharasutra* thread (1m long 10g) was extracted in to 250ml of methanol three times overnight. The Methanol extract was analyzed under the following conditions.

For TLC analysis plate with Silica gel 60 F254 TLC (Merck, Germany), 7X6 cm was cut with pair of scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC analysis. Applied sample volume 8-µl using the capillary at distance of 1 cm at 2 track, Methanol extract was tested in TLC for the presence of curcumin Standard solution was prepared using (0.1mg) of curcumin dissolved in 5ml methanol. Methanol extract of *ksharasutra* thread (5ml) was used for the study. The curcumin standard (8µl) and the *ksharasutra* sample (8µl) were spotted on the same plate, and develop the TLC fingerprint using the solvent system; Methanol: Chloroform 5: 95.Plate were later sprayed with methanol and chloroform reagents and the same

were placed in hot air oven for 100°C for 1 min for the development of color in separated bands. The developed TLC plates were air dried and observed under ultra violet UV light at both 254 nm and 366 nm. The movement of the analyte was expressed by its retention factor ($R_F$). Values were calculated for different sample as follow. Same procedure followed for the detection of Betaine.

**GC-MS analysis of *Ksharasutra***

GC-MS analysis was carried out to identify the Chemical components of *ksharasutra* and to find out the chemicals that responsible for the clinical success of the thread.

*Ksharasutra* thread (1m long 10g) was extracted three times with acetone (overnight, 250ml each). Acetone extract of a *Ksharasutra* threads were analyzed under the following condition.

All conditions and other parameters were set using the chemstaion software integrated with the instrument to get the optimum results of analysis Gas Chromatography/Mass selective Detector: Agilent 6890 series/Agilent 5973 N series. Capillary column: Rtx-5(crossbond 5% diphynil, 95% dimethyl polysiloxane) Column Elite-5MS fused silica capillary column (0.25mm ID, 30m, 0.25µmdf). Injection mode –splitless, splitb ratio, injection volume 2µl, injection temperature 230°C.Oven temperature was programed from 35°C (5 min hold) to 250°C (10 min hold) at 5°C/min, 250°C to280°C (10 min hold) at 5°C/min. Total run time was 74 min. and carier gas Helium constant flow 0.5L/min. EM voltage:1200. MS transfer line was 250°C. Scaned parameters were 15(amu)-550(amu). There was no solvent delay. Library search was using W9N08 database.

Interpretation on mass spectrum of GC-MS was done using the database of Institute Industrial Technology, having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known compounds stored in the library. The name, molecular weight and structure of the components of the test materials were ascertained.

**Physio-Chemical Parameters**

Physio-chemical parameters of the powdered drug such as ash values, extractive values loss on drying (moisture content) were performed following the method of Preliminary phytochemical screening performed by (Harborne, *et.al.*, 1999) [7]. pH was recorded by using pH meter.

## 3. OBSEVATION AND RESULTS

**Table 1: Results of Phytochemical constitution of *Ksaharasutra***

| Phytochemical Constituents | Result Present(+)/Absent(-) |
|---|---|
| Steroids | - |
| Alkaloids | + |
| flavonoids | + |
| Tannins | + |
| Glycoside | - |
| Saponins | + |
| Reducing sugar | - |
| Non reducing sugar | - |

The present study revealed that the *Ksharasutra* contains bioactive compounds. The phytochemical constituents were screened by qualitative methods and the results are presented in Table1.Accordingly, the brownish green color formation indicates the presence of Tannin. Similarly, the presence or absence of color change indicate positive and negative results (Table .1). The study showed that the positive results were obtained for, alkaloids, flavonoids, saponins and tannins while steroids, reducing sugars, sugars and cardiac glycoside gave negative results. (Table.1)

Physiochemical analysis of *ksharasutra* revealed that the total ash content was 12.32 %( w/w) and high water solubility indicates its clinical success, pH indicate high alkaline nature of *ksharasutra*. Loss on drying 26.34%( w/w) indicate its hyprogopic action, high ash content 66.4%( w/w)reported in *Ksharasutra* because of the *Kshara* was the main ingredient, acid solubility3.6%( w/w)while sulphated ash 3.6%( w/w) and acid insoluble ash remain 1.36%( w/w)(Table 2)

**Table 2: Physio-Chemicalparameters of the *Ksharasutra***

| Parameter | %(w/w) |
|---|---|
| Total ash | 66.4 |
| Acid insoluble ash | 1.35 |
| Water soluble ash | 10.22 |
| Sulphated ash | 3.6 |
| Water soluble extractive | 75.32 |
| Loss on drying | 26.34 |
| pH | 9.45 |

**Table .3: TLC of the *Ksharasutra* extract**

| $R_f$values and color of the standard | | $R_f$values and color of the *ksharasutrra* extract | |
|---|---|---|---|
| Before spraying | After spraying | Before spraying | After spraying |
| ʎ254mm & ʎ366mm | | ʎ254mm & ʎ366mm | |
| 0.57 curcumine | 0.57(Brilliant yellow) | 0.57 | 0.57(Brilliant yellow) |
| 0.69 curcumine | 0.69(Brilliant yellow) | 0.69 | 0.69(Brilliant yellow) |
| 0.82 curcumine | 0.82(Brilliant yellow) | 0.82 | 0.82(Brilliant yellow) |
| 0.45 Betaine | 0.45(white) | 0.45 | 0.45(white) |
| 0.57 Betaine | 0.57(white) | 0.57 | 0.57(white) |

Curcumin was obtain as a red-yellowish mixture, which was separated into pure compounds by repeated preparative TLC analysis and then it was. identified as cucumin by direct comparison with authentic samples**.** Guglesterone brown color and $R_f$0.38 and 0.46 and betaine$R_f$0.45 and 0.57 standard comply with the *Ksharasutrra* extract. The yellow ring of the TLC plate indicates the presence of Curcuminin *ksharasutra* extract,where as white ring indicate betaine and these three organic compounds may responsible for pharmacological value of the *ksharasutra.*(Table 3).

The GC-MS analysis of *ksharasutra* threadprepared by busing *Euphorbia Antiqurum* latex as binding agentextract pharmacologically important organic compounds were detected; antifungal and antibacterial activity3-Hexadecene and Benzene, 1-(1,5-dimethyl1-4hexynyl)-4-methyl,observed antibacterial activity.

**Table 4.: GC-MS Chromatogram of acetone extract of the *Kshartasutra*prepared by busing*Euphorbia Antiqurum* latex as binding agent**

| No. | RT | Name of the compound | Molecular formula | MW | Compound Nature | **Activity | Peak Area % |
|---|---|---|---|---|---|---|---|
| 1 | 4.29 | 6-Dodecene<br>3- Dodecene<br>5-Teteradecene | C12H24 | 168 | Aromatic compound | Antifungal/antibacterial | 4.17 |
| 2 | 6.12 | 2-pentanone,4-hydroxy-4-methyl<br>2-hexanone, 4methyl<br>1,3 Dixolane-2-methanol, 2,4-dimethyl | C6H12O2 | 116 | Nutrient compound | Nutritive | 73.75 |
| 3 | 7.36 | 3-Hexadecene<br>1-Hexadecanol<br>3-Teteradecene | C16H32 | 224 | Aromatic compound | Antimicrobial | 5.45 |
| 4 | 10.72 | 1-Hexadecanol<br>Hexadecen-1-0l, trans-9-<br>5-Octadecene | C16H34O | 242 | Aromatic compound | Pesticide | 4.56 |
| 5 | 23.86 | Ar-tumerone<br>Benzene, 1-(1,5-dimethyl1-4hexynyl)-4-methyl<br>Benzenebutanal, c4-dimethyl- | C15H20O | 290 | Aromatic compound | Antimicrobial | 3.8 |

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

Interpretation on mass spectrum of GC-MS was done using the database of the Institute of Industrial Technology, Colombo, having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known compounds stored in the database. The name, molecular weight and structure of the components of the test materials wereascertained.The nine major phytochemical constituent's mass spectra were identified in *ksharasutra*; 6-Dodecene,3-Hexadecene and Benzene,1-(1,5-dimethyl1-4hexynyl)-4-methyl.Pharmacologically analysis, 2-methoxy- 4-vinylphenol had anti-inflammatory activities while other chemical components possess antimicrobial activities (Table 4.)

*Ksharasutra* therapy of fistula seems to make good use of chemical cauterization with this medicated thread.

The ingredients of the thread are natural mild herbal base which perform 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. *C.longa* powder is a reputed drug for its antiseptic, antimicrobial and antihistamine action by which it counter act any kind of local irritation reaction. In addition strong alkaline property and high calcium content one of the important inorganic element of ash *Achyranthes aspera.*

**Effect of pH on the therapeutic success**

The knowledge of the pH of chronic wounds and further studies on the specific effect of pH could therefore constitute a crucial factor in the future treatment of chronic wounds. *Kshara* as well as *Ksharasutra* posses' alkaline environment pH 9.54 (Table 2). Glinz et *al*., 1971describes that pH values below the granulation tissue therapy compresses saturated with NaCl from 7.4 to 8.2 as a muchmore favorable for the complete healing of 90% of the transplants than any other pH ranges.

Fistula wounds are often colonized with endogenous fecal, oral and dermal micro-organisms. Most staphylococci enzymes in alkaline environments are less active, for example through in to the application of maggots to improve wound healing by a shift in the wound environment in alkaline. Similarly *kshrasutra* highly alkaline in nature, these designated as wounds alkalization, or even one bacteriolytic or bacteriostatic effect.

From the above analysis it is evidentthat the usefulness of *Ksharasutra* is due to the presence of alkaline chemical compounds in the threadand their therapeutic values i,e anti-inflammatory antifungal, antiseptic and antibacterial property. It is strongly believed that detailed information as presented in this text on the phytochemicals and their various pharmacological aspects view a modern scientific validation for the Ayurvedic parasurgical treatment procedure of *ksharasutra*

## 4. CONCLUSION

From the above analysis it is evident that the usefulness of Flex thread (*Ksharasutra)* is due to the presence of chemical compounds present in the device and their therapeutic values i,e anti inflammatory, antifungal, antiseptic and antibacterial property. It is strongly believed that detailed information as presented in this text on the phytochemicals and their various pharmacological aspects gives a scientific validity of the *ksharasutra* treatment

## 5. REFERENCES

1. Goligher J C, Elllis A, Pissidis A G, A critique of anal glandular infection in the etiology and treatment of idiopathic anorectal abscess and fistulas, Br. J. Surg 1967; 54: 977-983.
2. Keighley MRB & Fielding JWL. Management of faecal incontinence and results of surgical treatment. Br J Surg 1983; 70: 463-468.
3. Kennedy HL and Zegarra JP. Fistulotomy without external sphincter division for high anal fistulae. Br J Surg 1990; 77: 898-901.
4. Sushruta; Chikitsa sthana-Bhagandara chikitsa; Sushruta Samhita, 5th Edition, 1994 Motilal Banarasidas, New Delhi 18th Ch. Pg. 421-425.
5. Sushruta; Nidana sthana-Bhagandara nidana; Sushruta Samhita, 5th Edition, 1994 Motilal Banarasidas, New Delhi. 4th Ch. Pg. 241-242.
6. Sushruta; Nidana, Sharira, Chikitsa sthana; Sushruta with Nibandha sangraha vyakya of Dalhana and Nyaya chandrika Panjika of Gayadasachrya, 6th Edition, 1997, Chowkhamba Orientalia, Varanasi.

7. Sushruta; Chikitsa sthana-Bhagandara chikitsa; Sushruta Samhita, 5th Edition, 1994 Motilal Banarasidas, New Delhi 18th Ch. Pg. 421-425.
8. Tagart REB (1966): the anal canal and rectum: Their varying relationship and its effect on anal continence. Dis Colon Rectum 9, 449.
9. Taniguchi S, Yamanari H, Inada K el. Adenocarcinoma in the anal canal associated with a fistula: report of a case. Surg Today, Jpm J Surg 196; 926: 707
10. Van Tets WF & Keighley JC. Continence disorders after fistulotomy. Dis Colon Rectum 1994; 37: 1194-1197.
11. Trease, GE, and Evans, phytochemical screening in: Textbook of pharmacology, 10th Edn, bailiere Tindal Ltd, London, (1989), pp;541.
12. Shanmugam, studies on the comparison of phytochemical constituents and antimicrobial activity of Curcuma longa verities, International Journal of current microbiology and Applied Science 2014; 03: 573-581.

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