**PRILIMIUNARY PYTOCHEMICAL ANALYSIS AND THIN LAYER
CHROMATOGRAPHY OF KSHARASUTRA**

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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 nature the 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 and TLC tests were carried out to identify the chemical compounds in the thread. A good number of chemical present in the *ksharasutra* like Euphol, which are possess activities like anti-inflammatory, antibacterial & antifungal, Water soluble ash of *Achyramthus aspera* (whole plant) contains Betaine. Pharmacologically rhizome of *Cucuma longa* possesses anti-inflammatory, antibacterial, antifungal, anti-infective, analgesic and anti puritic effect is due to the presence of active constituents Curcumin and, Betaine

KEYWORDS: *Ksharasutra*, fistula in ano, chemical analysis.

INTRODUCTION

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 filtered 21 times (Su.Su.11/11) and finally, the *kshara* is obtained by evaporating the filtered solution Latex (*Euphorbia Antiquorum*) is collected by giving perpendicular incision the stem of *Euphorbia Antiquorum* 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 Antiquorum*), *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 Antiquorum* latex, this process is repeated for 11 days. On the 12th day the thread is again smeared with *Euphorbia Antiquorum* 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 19th day the dried thread is smeared again with *Euphorbia Antiquorum* 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 Antiquorum* latex. *Ksharasutra* is one of the chief treatment modality in the management of fistula in ano. Main objectives of this study; to identify the chemical composition of *Ksharasutra* were prepared according to the classical method.

MATERIAL AND METHODS

Ksharasutra extract

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

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% ammonia solution was added to the extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

Test for cardiac glycosides

Approximately 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 acetic acid containing 1 drop of $FeCl_3$. The above mixture was carefully added to 1ml of concentrated H_2SO_4 . Presence of cardiac glycosides was detected by the formation of a brown ring.

Test for Alkaloids

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

Test for Glycosides

The extract of 1.00ml in a boiling tube was mixed with 10ml. of 50% H_2SO_4 . 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

To a 3.00 ml of test solution was added with a 2 ml of Fehling's reagent and 2.00 ml of water. Formation of reddish orange color indicates the presence of reducing sugar.

Sugars

3.00 ml of the test solution was added with very small quantity of anthrone reagent and a few drops of concentrated H₂SO₄ and heated. Formation of green or purple color indicates the presence of sugars.

Identification of Curcumin, Betaine and Euphol in *Ksharasutra*

Curcuma longa was 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 Euphol also prominent pharmacologically important compounds available in *Ksharasutra* Hence the TLC method was carried out to identify the curcumin, Betaine and Euphol in *ksharasutra*. Pre preparation of samples for TLC analysis.

TLC analysis of *Ksharasutra*

Ksharasutra thread (extract 10g) was extracted in to 250ml of methanol three times overnight. The Methanol extract was analyzed under the following conditions. Separation of curcumin by TLC using different solvent System: 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- μ l 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 (8 μ l) and the *ksharasutra* sample (8 μ l) were spotted on the same plate, and develop the TLC fingerprint using the solvent system; Methanol: Chloroform 5: 95. Plate was later sprayed with methanol and chloroform reagents and the same was placed in hot air oven for 100oC 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 (RF). Values were calculated for different sample as follow. Same procedure followed for the detection of Betaine and Euphol.

RESULTS

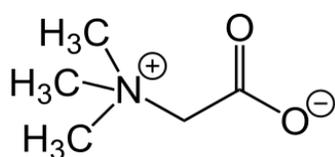
Table 1: Results of Phytochemical constitution of *Ksaharasutra*.

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

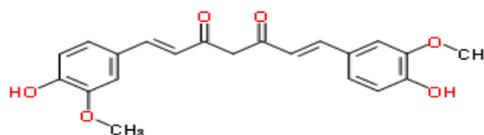
The study showed that the positive results were obtained for, alkaloids, flavonoids, saponins and tannins while steroids, reducing sugars, sugars and cardiac glycoside gave negative results.

Table 2: TLC of the *Ksharasutra* extract.

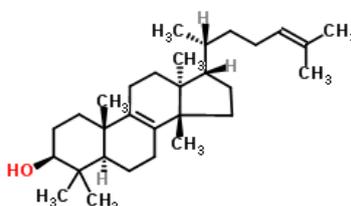
R _f values and color of the standard		R _f values and color of the <i>ksharasutra</i> extract	
Before spraying	After spraying	Before spraying	After spraying
λ254mm & λ366mm		λ254mm & λ366mm	
0.57 curcumin	0.57(Brilliant yellow)	0.57	0.57(Brilliant yellow)
0.69 curcumin	0.69(Brilliant yellow)	0.69	0.69(Brilliant yellow)
0.82 curcumin	0.82(Brilliant yellow)	0.82	0.82(Brilliant yellow)
0.38 Euphol	0.38(Brown)	0.38	0.38(Brown)
0.46 Euphol	0.46(Brown)	0.46	0.46(Brown)
0.45 Betaine	0.45(white)	0.45	0.45(white)
0.57 Betaine	0.57(white)	0.57	0.57(white)



Betaine



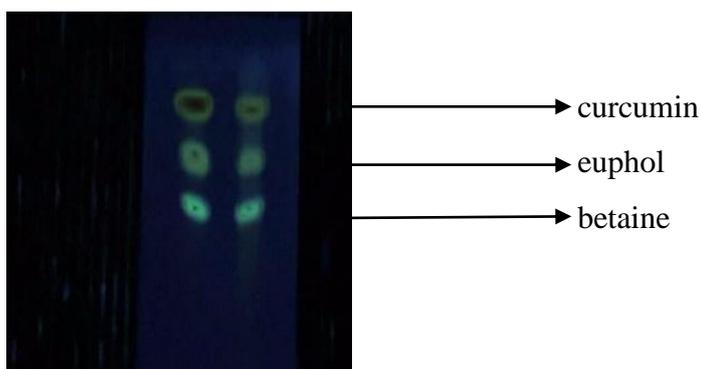
Curcumin



Euphol

Curcumin was obtained as a red-yellowish mixture, which was separated into pure compounds by repeated preparative TLC analysis and then it was identified as curcumin by direct comparison with authentic samples. Euphol brown color and R_f 0.38 and 0.46 and betaine R_f 0.45 and 0.57 standard comply with the *Ksharasutra* extract.

The yellow ring of the TLC plate indicates the presence of Curcumin in *ksharasutra* extract, where as brown ring indicates Euphol, white ring indicate betaine and these three organic compounds may responsible for pharmacological value of the *ksharasutra*.



TLC plate indicates curcumin, euphol and betaine

DISCUSSION

Medicinal and healing properties of *ksharasutra* are closely related to their chemical components which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins, tannins etc., The present study revealed that *ksharasutra* contains bioactive compounds. (The phytochemical constituents were screened by qualitative methods and the results were presented in (Table 1). In these screening, the positive results were obtained for alkaloids, flavonoids, saponins, tannins and amino acids while negative results were given for triterpenes, steroids, sugar and reducing sugar.

Flavonoids found in *ksharasutra* that are ubiquitous in photosynthesizing cells and are commonly found in medicinal plants. They are known to be synthesized by plants in response to microbial infection, and that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms (Cowan *et al.*, 1999). Flavonoids are hydroxylated phenolic substances that occur as a C6-C3 unit linked to an aromatic ring. Their activity is probably due to their ability to complex formation with extracellular and soluble proteins and to make complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes (Cowan *et al.*, 1999).

Alkaloids have wide range of pharmacological activities including anti-bacterial activity. Tannins are also used as antiseptic and this activity is due to the presence of phenolic group. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of enteric diseases like diarrhea, dysentery, flatulence etc.

The mode of antimicrobial action of phytochemicals have been suggested by researchers Kris *et al.*, 2002, opinioned that phytochemicals may act by inhibiting microbial growth, inducing cellular membrane perturbations, interference with certain microbial metabolic processes, modulation of signal transduction or gene expression pathways. The presence of tannin in the *ksharasutra* implies it may have astringent properties and in addition, could quicken the healing of wounds and burns. This justifies their usage in fistula in ano (Dev *et al.*, 1968).

Curcumin is one of the major organic compound found in *ksharasutra* extract according to TLC observation (Table 2) Curcumin possess wide range of pharmacology activities. In *vitro*, curcumin exhibits anti- parasitic, anti- spasmodic, anti-inflammatory and anti-gastro intestinal effects.

The inhibition activity of carcinogenesis and cancer growth has been shown in parental and oral application in animal models. (Aruajoc *et al.*, 2000) The highlights of chemical structural features associated with the biological activity of curcumin are the o-methoxy phenol group and methylenic hydrogen that are responsible for the antioxidant activity of curcumin.

Curcumin (diferuloylmethane) enhanced cutaneous wound healing that showed earlier re-epithelialization, improved neo-vascularization, increased migration of various cells including dermal myo-fibroblasts and macrophages into the wound bed and a higher collagen content. (Sidhu *et al.*, 1999). Transforming growth factor beta-1 is also known to enhance wound healing, it may be possible that transforming growth factor- beta -1 plays an important role in the enhancement of wound healing by curcumin. (Sidu *et al.*, 1999, Singh *et al.*, 1998). It's antimicrobial (Shanmugam, et al., 2014), anti-inflammatory. Anti-oxidant and wound healing activities play vital role in clinical success of the *ksharasutra* for the treatment of fistula in ano patients.

The main chemical constituents which are responsible for proteolytic action of snuh i(Latex) was euphol (Anjaneyylulu et al., 1965). Betaine possess antibacterial, antifungal and wound healing activity (Vasudevamurthy et al., 2009).

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

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