Ksharsootra - a herbal medicated thread for the treatment of fistula-in-ano is being used in various Ayurvedic hospitals and dispensaries. Its efficacy has been well established through multicentric clinical trials conducted by ICMR. Therefore an effort has been made here to standardize Ksharsootra for which a complete analytical profile has been developed.

The standardization of plant based remedies, especially polyherbal remedies like Ksharsootra pose serious problems. Hence it was considered worthwhile to standardize not only the finished product Ksharsootra but also the raw material viz., thread, latex of *E. neriifolia*, Apamarg Kshar and turmeric used in the process. Keeping this in view, well authenticated raw material were procured.

Phytochemical studies were carried out on the latex of *E. neriifolia* and rhizomes of *C. longa* for the isolation of marker compounds. The hexane extract of *E. neriifolia* on column chromatography yielded four terpenoidal compounds. Two of these are novel compounds not reported so far in the literature and these are 11-Methoxy-6,7,12-triacetoxy-1(10),13-clerodadiene-3-one (K002) and 24-Methylene-3,12-dihydroxy-9,19-cycloartane (KO03). The third compound isolated was 3,25-Dihydroxy-9,19-cycloart-23-ene (KO04). This compound hitherto has not been reported from the latex of *E. neriifolia*. Euphol (KO01) was isolated using repeated column chromatography (by suction) and finally purified using reverse phase HPLC with acetonitrile as mobile phase. Euphol is the major bioactive triterpenoid from the latex and hence was used as a marker compound for the standardization of Ksharsootra. Curcumin (KO05), a well known major bioactive compound was isolated from the methanolic extract of the powdered dried rhizomes of *C. longa*.

The process of manufacturing Ksharsootra was systematically developed. Besides, the process of preparation of Kshar from *A. aspera*, the procedure and the
time of collection of latex was standardized. The process of giving eleven coating of latex, seven alternate coatings of latex/Kshar and three alternate coatings of latex/turmeric and finally drying, packing and sealing of Ksharsootra was standardized and all possible steps were undertaken to get Ksharsootra of uniform quality. Protocols for the standardization of Ksharsootra and its raw material were designed and reproduced as below:

**Ksharsootra**

1. Minimum breaking load of thread after removal of coated material. MBL for each thread ≥ 3.0 kg. Mean MBL ≥ 4.9 kg.
2. Length: 28.5 - 31.5 cm.
3. Mean diameter: 1.25 - 2.19 mm; at least two thirds of diameters fall in the range 1.56 - 1.88 mm.
4. Total weight: 0.9 - 1.1 g.
5. Weight of the coated material: 0.72 - 0.88 g.
7. Sulphated ash of the coated material (IP 1985 method): ≤ 94.8 % w/w.
8. Water soluble extractives of coated material (Hot * method): ≥ 74.3 %w/w.
9. Hexane soluble extractives of coated material (Hot * method): ≥ 2.5 %w/w.
10. pH of 1% w/v aqueous solution (Using digital pH meter): 9.6 - 10.8.
11. Sodium content (Flame photometry): ≥ 0.8 %w/w.
12. Potassium content (Flame photometry): ≥ 20.7 %w/w.
13. Total alkalis estimated as carbonate: N/25 HCl consumed per 100 mg of the coated material; end point pH 3.6 (using digital pH meter): ≥ 19.8 %w/w.
14. Turmeric content (Spectrophotometric method): ≥ 2.5 %w/w.
15. Curcumin content (Prep-TLC-spectrophotometry): ≥ 0.011 %w/w.
16. Euphol content (Prep -TLC -spectrophotometry): ≥0.56 %w/w.
17. TLC densitometric profile (Densitometric scanning).

**Raw material**

**Flax thread**
1. Minimum breaking load. MBL for each thread ≥3.7kg. Mean MBL ≥5.2kg.
2. Mean diameter : 0.33 - 0.60 mm; atleast two thirds of diameters fall in the range 0.42 - 0.51 mm.
3. Mean weight per unit length : 2.16 - 2.64 mg.

**Latex**
1. Loss on drying (IP 1985 method) : ≤5 %w/w.
2. Sulphated ash (IP 1985 method) : ≤23.5 %w/w.
3. Ethanol soluble extractives (Hot method\*): ≥42.4 %w/w.
4. Hexane soluble extractives (Hot method\*): ≥45.2 %w/w.
5. Euphol content (Prep-TLC spectrophotometric method): ≥4.8 %w/w.

**Kshar**
1. Loss on drying (IP 1985 method) : ≤5 %w/w.
2. pH of 1% w/v aqueous solution (Using digital pH meter) : 10.6 - 11.7.
3. Total alkalis estimated as carbonates : Volume of N/25 HCl consumed per 100mg; end point pH 3.6 (using digital pH meter) : ≥24.7 %w/w.
4. Sodium content (Flame photometry) : ≥1.5 %w/w.
5. Potassium content (Flame photometry) : ≥33.7 %w/w.

**Turmeric**
1. Loss on drying (IP 1985 method) : ≤5 %w/w.
2. Sulphated ash (IP 1985 method): ≤18.2%.
3. Ethanol soluble extractive (Hot method\textsuperscript{*}): ≥7.0 %w/w.
4. Hexane soluble extractive (Hot method\textsuperscript{*}): ≥1.03 %w/w.
5. Curcumin content (Prep-TLC spectrophotometric method): ≥0.27 %w/w.

Note: Hot method of extractive is as outlined in section 2.4.8.

MBL = Minimum breaking load.

Methods for the estimation of marker compounds were systematically developed. The three steps involved, here were optimization of extraction procedures, preparation of calibration plots and validation of the assay. The extraction procedures were also developed systematically ensuring complete extraction of the marker compound. Calibration plots were developed to find the linear range for determining marker in the test material. Validity of the assay procedures was assessed by finding out the recovery of known amounts of reference marker added to the test material. Special emphasis, on the simplicity of the methods used, was laid while developing them so that they could even be used by small scale manufacturers.

Analysis of 10 batches of clinically efficacious Ksharsootra and the raw material used therein was carried out. This involved analysing a total of 50 Ksharsootra (5x10) and 30 samples for each of the raw material (3 x10). The results obtained were statistically analysed and limits were proposed for each of the parameters used for standardization. On the basis of limits drawn, pharmacopoeial monographs (Appendix I) on Ksharsootra and its raw material were prepared.

In-process quality control studies were also carried out on Ksharsootra and eleven parameters were assessed after successive coatings on to the thread.
life studies were carried on Ksharsootra to determine its expiry date. Here estimations of curcumin and euphol were carried out using TLC-densitometric method instead of the prep-TLC spectrophotometric method as it is faster and more sensitive. TLC-densitometric methods also involved the three steps i.e., the development of extraction procedure, preparation of calibration plots and validation of the assay. Initially pilot accelerated stability study was carried out for the selection of appropriate temperatures and suitable parameter for stability studies. Consequently the temperature ranges selected were 40°, 45° and 50°C and the parameter selected for the planned accelerated stability studies was curcumin content. The shelf life calculated from the accelerated stability studies was around 15 years at 25°C.