INTRODUCTION
1.1 ANAL FISTULA

Anal fistula, haemorrhoids, proctalgia fugax, hypertrophied anal papilla and pruritus ani are some of the diseases of the ano rectal region\(^1\). The ano rectal fistulae form quite a large share of all diseases of this part of the body. It is considered second in importance to haemorrhoids among all ano rectal abnormalities and is prevalent all over the world. As per study in a London hospital\(^2\) it was reported to be 10% of all in-patients and 47% of all new out-patients. A similar study from India\(^3\) reported anal fistulae to constitute 1.6% of all surgical admissions.

Fistula-in-ano is characterized by single or multiple sinuses with purulent discharge in the perianal area. Though benign in nature fistula-in-ano leads to major physical, psychological and social problems due to persistent discharge through the external opening\(^4\). The medical profession, therefore has always been on alert to devise and provide procedures and methods surgical or otherwise which could control the disease effectively. It is a disease for which operative procedures have been advocated and practised by surgeons at various times. Surgical treatment requires familiarity of the surgeon with the anatomy of the ano rectal region. The treatment usually comprises laying open or completely excising the fistulous track and then allow healing by secondary intention. The patient has to endure the most discomforting aspect of surgery in the form of wound infection and post operative dressings. Complete healing takes from few weeks to months with rather high chances of recurrence\(^5\). Misra and Kapoor\(^6\) have reported a non operative treatment for fistula-in-ano. It involves insertion of a braided stainless steel wire at the primary out-patient department.
1.2 KSHARSOOTRA - NON SURGICAL REMEDY FOR ANAL FISTULA

In Ayurvedic practice fistula-in-ano is treated without surgery employing Ksharsoota described by the ancient Indian surgeon Sushruta in his famous treatise Sushruta Samhita 600 B.C. (Fig.1).

The technique involves insertion of a specially prepared alkaline medicated thread "Ksharsoota" coated with herbal products into fistulous track and tied with knots. The thread cuts through the fistulous track in due course of time to lay open the wound which exhibits spontaneous healing. Deshpande and Sharma used this non operative Ayurvedic procedure for the treatment of fistula-in-ano. Sharma

Fig 1: Original reference on Ksharsoota in Sushruta Samhita (600 BC);
Chikisthanam : Chapter 17 : Shlokas 29-33.
et al\textsuperscript{10} have also discussed this approach for the treatment of anal fistula. Varshney et al\textsuperscript{11} obtained promising results in treating anal fistula with Ksharsootra.

Recently the results of chemical analysis of the Ksharsootra thread has been reported\textsuperscript{12}. They employed the latex of \textit{Euphorbia antiquorum} instead of \textit{Euphorbia neriifolia} in their preparation which is different from actual Ayurvedic formulation. A preliminary attempt to standardize Ksharsootra has been made by Dwivedi \textit{et al}\textsuperscript{13}. Ksharsootra contains three basic ingredients viz., Snuhi Ksheer i.e., latex of \textit{Euphorbia neriifolia}; Kshar a specially prepared alkaline powder from the ash of \textit{Achyranthes aspera} (Apamarg) and Haridra i.e., turmeric powder from the dried rhizomes of \textit{Curcuma longa}. The thread is given eleven coatings of the latex followed by seven alternate coatings of Kshar/latex and finally three alternate coatings of turmeric/latex.

1.3 \textbf{LITERATURE REVIEW ON THE PLANTS USED IN THE PREPARATION OF KSHARSOOTRA}

1.3.1 \textit{Euphorbia neriifolia}

\textit{Euphorbia neriifolia} L. (fam. Euphorbiaceae) is a large erect glabrous shrub upto 20 ft. or more, common in rocky places throughout the Western Peninsula cultivated elsewhere in India. It is known as Patrasnuhi or Snuhi in Sanskrit\textsuperscript{14}.

The plant yields a copious milky latex. It is reported to contain euphol and its 3α-epimer\textsuperscript{15}. Not much work has been done on the latex of \textit{E. neriifolia}, though other parts like roots, stems etc. have been exhaustively worked up. Major constituents reported from the plant include terpenoidal compounds. Baslas \textit{et al}\textsuperscript{16} isolated 12-deoxy-4β-hydroxyphorbol-13-dodecanoate-20-acetate,euphol, euphorbol hexacosanoate, \textit{n}-hexacosanol and 24-methylene cycloartenol from the bark of \textit{E. neriifolia}. The same group\textsuperscript{17} later reported the presence of 12-deoxyphorbol-13,
20-diacetate, delphinidin-3,5-diglucoside, tulipanin-3,5-diglucoside, pelargonin-3,5-diglucoside from various parts of the plant. Other triterpenes and diterpenes isolated from the plant include friedelan-3α- and 3β-ol, taraxerol, glut-5(10)-en-1-one, antiquorin, nerifolene, jolkinolide A(ent-8α,14α-epoxyabieta-11,13(15)-dien-16,12-olide). Quantitative GLC method for phorbol and related diterpenes from Euphorbia spp. has been developed by Kinghorn et al. Two ellagic acid glycosides have been reported from E. neriifolia.

E. neriifolia is a reputed Ayurvedic medicine. The latex enjoys reputation as remedy for snake bite and scorpion sting. It has been reported to show a low grade anticoagulant activity and normal fibrinolysis in rabbit plasma.

Hartwell has reported anticancer activity attributed to the presence of 12-deoxy-phorbol-13,20-diacetate and ingenol triacetate. Euphol, a major triterpenoid from E. neriifolia on administration was found to exhibit hypotensive activity. Latex has been found to be very toxic to certain plant parasitic nematodes.

1.3.2 *Achyrantes aspera*

*Achyrantes aspera* L. (fam. Amaranthaceae) is popularly known as Apamarg in Sanskrit and Latiira in Hindi. It is found growing wild, all over India.

A widely occurring phytoecdysone; ecdysterone has been isolated from *A. aspera*. It contains traces of basic substances one of which has been identified as betaine. The alkaloidal contents have been found to be maximum during the fruiting stage in roots and shoots. Oleanolic acid has been reported from *A. aspera*. Hydrocarbons and their derivatives isolated from the plant include hentriacontane, 10-octacosanone, 10-triacosanone, pentatriacontane,
Iririaconitine, hexatriaconan, 4-irilriacontane, 6-pentatriacontane, 36,47-dihydroxyhexentacontan-4-one, tricontanol, 27-cycloheptacosan-7-ol and 16-hydroxy-26-methylheptacosan-2-one. Saponin glycosides such as saponin A[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl (1→3) oleanolic acid], saponin B[β-D-galactopyranosyl(1→28) ester of saponin A], saponin C[β-D glucopyranosyl ester of α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl(1→3)oleanolic acid] and Saponin D[β-D-glucopyranosyl ester of α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl(1→3)oleanolic acid]. The plant contains appreciable quantities of Zn, Ca, Mg, Mn and Cu. Presence of folic acid proteins and vitamins A,B,C have been also reported.

The plant plays important role in etnomedicine for varied medicinal properties. It is used singly or in combination with other drugs in angina, cardiac failure, and cholesterolinemia. A. aspera possesses significant diuretic activity. Achyranthine and the saponin fraction from A. aspera have been observed to exert diuresis when tested on albino rats. The saponin mixture has been reported to exert cardiac stimulant activity. The plant is reported to produce hypoglycaemic effect in normal as well as in diabetic animals.

Polyherbal drugs containing A. aspera are claimed to be useful in experimentally induced hepatopathy in sheep. It exhibited beneficial effect in the treatment of dysmenorrhea. Non-polar fraction of A. aspera has shown abortifacient activity. It was also found to prevent pregnancy and showed anti-implantation activity. A. aspera possesses pronounced insect moulting hormonal activity and is useful in cosmetics and dermatological composition or keratinocyte culture medium. Acute toxicity test on the alcoholic extracts.
dose of 5-6 mg/kg) showed significant depletion in respiration and loss of reflexes\textsuperscript{36}.

1.3.3 \textit{Curcuma longa}

The rhizome of \textit{Curcuma longa} L. (fam. Zingiberaceae) has been used as a medicine, condiment and colouring agent for thousands of years. The plant popularly known as Haldi in Hindi and Haridra in Sanskrit\textsuperscript{68} is extensively cultivated all over India. The rhizomes of \textit{C. longa} are a rich source of essential oils\textsuperscript{69,70}. Fang \textit{et al}\textsuperscript{71} have reported limonene, $\alpha$-pinene, linalool, caryophellene and about 20 other compounds from its essential oil. Besides the above mentioned compounds camphene, terpienene, curcumene, turmerone, $ar$-turmerone, borneol, iso-borneol, camphor, eugenol, cineole, curdione, curzerenone and other diarylheptanoids as curcumin, monomethoxycurcumin and didemethoxycurcumin have been identified in the volatile oil of \textit{C. longa}\textsuperscript{72}. Several sterols and fatty acids including campesterol, stigmasterol, $\beta$-sitosterol, cholesterol, saturated straight chain and saturated isomonoenoic and dienoic fatty acids have been reported from the rhizomes of \textit{C. longa}\textsuperscript{73}.

Curcuminoids are the major phenolic constituents of \textit{C. longa} and various methods for their isolation have been reported\textsuperscript{74,75}. The main bioactive curcuminoid is curcumin for which many improved methods of isolation have been evolved\textsuperscript{76-84}. The other curcuminoids isolated include demethoxycurcumin, bisdemethoxycurcumin\textsuperscript{78,79}, cyclocurcumin\textsuperscript{85}. A biosynthetic pathway for curcumin and related diarylheptanoids has been proposed\textsuperscript{86,87}. Curcumin content is reported to be highest in \textit{C. longa} among the various \textit{Curcuma} spp.\textsuperscript{88}, out of the two \textit{C. longa} varieties it is more in bulb type than in finger type\textsuperscript{89}. Dihydrocurcumin is also reported from the rhizomes of \textit{C. longa}\textsuperscript{90}. Mimura \textit{et al}\textsuperscript{91}
later developed a method for the synthesis of tetrahydrocurcumin. Sesquiterpenoids reported from turmeric include \( \alpha \)-turmerone, \( \beta \)-turmerone, \( \alpha \)- and \( \beta \)-turmerone, curone, (4S,5S)-(+) germacrone, \( \beta \)-sesquiphellandrene, turmeronol A, turmeronol B, germacrone-13-al, 4-hydroxy-bisabola-2,10-diene-9-one, \( \alpha \)- and \( \beta \)-turmerone, turmeronol A, turmeronol B, germacrone-13-al, 4-hydroxy-bisabola-2,10-diene-9-one, 2,5-dihydroxybisabola-2,10-diene, procumbradiol, curcumeneone, dehydrocurdione, bisabola-3,10-diene-2-one, bisacumol, bisacurone, curcumeneol, iso-procurcumeneol, zedoarondiol, procurcumeneol, epiprocucumenol and 4,5-dihydroxy-bisabola-2,10-diene. The sesquiterpenoid composition has been used to classify *C. longa* into two chemotypes. Four polysaccharides viz., Ukonan A, Ukonan B, Ukonan C and Ukonan D have been isolated and characterized from the rhizomes of *C. longa*.

Qualitative methods to check adulteration in *C. longa* by other *Curcuma* spp. have been reported. Various spectrophotometric methods have been used for the estimation of total curcuminoids and curcumin in *C. longa*. Other quantitative methods for the estimation of curcumin include spectrofluorimetry, supercritical fluid extraction, TLC densitometry and HPLC. Trace elemental analysis of *C. longa* has been carried out using atomic absorption spectrophotometry and neutron activation analysis. *C. longa* has been reported to contain large amounts of chromium. Lead chromate has been detected as adulterant in some commercial samples of turmeric. Methods for the detection and determination of lead have been reported.

From times immemorial turmeric finds an important place in the Chinese and Indian traditional systems of medicine including...
Ayurveda and Unani. It possesses significant antibacterial, antifungal, hepatoprotective, antitumor, anti-inflammatory, hypolipidaemic and antithrombotic activities. Turmeric is known to possess anti-inflammatory activity. Curcumin also shows anti-inflammatory activity though it has been found to be less active than sodium curcuminate or ibuprofen. Curcumin is reported to be a potent inhibitor of leukotriene/prostaglandin biosynthesis which may be at least in part responsible for anti-inflammatory activity of curcumin/C. longa.

Skin wound healing property of ointment containing turmeric has been reported by Kumar et al. Different extracts of turmeric are reported to exert antioxidative activity. The naturally occurring curcuminoids inhibit lipid peroxidation and protect cell damage by active oxygen. It is observed that curcumin has a distinct hypocholesteremic effect in induced hypercholesteremic rats. Several groups have demonstrated the bile secreting effect of curcumin. C. longa is also claimed to possess significant hepatoprotective activity and caused reversal of severe fatty changes caused by carbon tetrachloride. Controversial data exist regarding the antiulcerogenic activity of curcumin. A marked decrease in mucin secretion and ulceration has been reported with curcumin and (4S,5S)-(+)–germacrone, 4,5-epoxide. Bhatia et al. in contrast did not find any protective action of curcumin against histamine induced gastric ulceration. C. longa has been reported to possess antibacterial, antifungal, insecticidal, antimicrobial, nematocidal, anticancer, tumor reducing, antimitogenic, antiplatelet aggregation, antifertility and antidiabetic activities. C. longa has been employed in herbal eye formulations.
drop preparations, useful in the treatment of conjunctivitis and various other eye ailments. Polysaccharides from *C. longa* have been reported to show a reticuloendothelial system potentiating activity. It is found to be a major ingredient in various herbal cough syrups found to be effective in acute cough and bronchial asthma. Turmeric has been employed in a variety of skin cosmetics. A number of acute and subacute toxicity studies have been carried out with turmeric. Neither turmeric nor its extracts have been reported toxic even at very high concentration. Howsoever the oral LD50 value for the petroleum ether extract in rats is reported to be 12.2g/kg b.w. Turmeric oleoresin does not show any chronic toxicity whereas ethanolic extract of *C. longa* induced significant changes in heart and lung weights of mice.

**1.4 STANDARDIZATION OF PLANT BASED REMEDIES**

The traditional systems of medicine which were practised by Charaka and Sushrutta are returning to the forefront with its increasing popularity. It has been observed that 80% of the World population depends on traditional and indigenous medicines for their primary health care needs. The medicines used in various systems of traditional therapeutics viz., Chinese, Indian and European are derived mainly from plant and plant based products. The world health organisation also encourages, recommends and promotes traditional/herbal remedies in National health care programmes because such drugs are available at low cost. These are comparatively safe and people have faith in such remedies. Despite the ever increasing global interest in plant and plant based drugs, these have not come at par with the modern or so called allopathic drugs. The reason being, the constancy of composition of plant based drugs as compared to allopathic...
drugs has not yet been achieved mainly because of lack of standardization and quality control of plant based remedies.

In this era of quality boom, it becomes all the more necessary to have products of consistent quality, a fact equally highlighted by the ISO 9000 revolution. The WHO expert committee also observed the need to develop protocols for quality control of plant material because of increasing demand and international trade in traditional medicines/natural products\(^2\).

Standardization problem arises from the complex composition of drugs which are used in the form of whole plant, part of plants and of plant extracts. Inconsistency may be due to several factors such as age and origin, stage of harvesting, method of drying and so on. Thus standardization of a drug does not include only analytical control but requires also a thorough description of the starting material including basic ingredients of quality. The analytical control could be on the basis of therapeutically active constituent (where known) or on the basis of chemical markers where therapeutically active constituents are not known\(^\footnote{233}\). Thus to ensure therapeutic efficacy of the drug it is necessary to have standardization in terms of biological, chemical and physico-chemical parameters.

General protocols for the standardization of raw materials for plant and plant based formulations include authentication by detailed morphological, taxonomical studies, organoleptic evaluation, microscopical examination including quantitative microscopy, volatile matter, ash value, extractive value, chromatographic profile and quantitative estimation of a marker compound by various quantitative methods.

Different methods used for the standardization of herbal drugs are spectrophotometry\(^\footnote{111-114}\), fluorimetry\(^\footnote{115}\), polarography, titrimetry, gravimetry and
chromatography. The chromatographic methods include TLC\textsuperscript{234-237}, HPTLC finger print profile\textsuperscript{110}, HPLC\textsuperscript{234,238-244}, GLC\textsuperscript{245-248} etc. Each technique has its own merits and demerits with respect to applicability, accuracy, precision and reproducibility of the assay. Detection and determination of foreign matter such as soil, stones, insects, animal excreta and other non drug plant materials, pesticide residue, heavy metals\textsuperscript{249}, microbial and radioactive contaminants has become necessary.
1.5 RESEARCH ENVISAGED

Ksharsootra technique for the treatment of anal fistula is known since 600 B.C. but every practitioner has been making his own material. There has been no uniformity of the method of preparation and as a consequence the finished product has been used without being tested for any parameters of standardization and quality control. It was therefore, considered extremely, important to evolve standardized procedure of manufacture and to devise appropriate parameters for standardization and quality control for both the raw material as well as for the finished product. Various batches of Ksharsootra were prepared by standardizing the raw material and the batches prepared by standardized method of manufacture were inducted into clinical trials. It was considered worthwhile to evolve physico-chemical parameters of standardization and quality control of these batches of Ksharsootra, found clinically efficacious so that such parameters for the raw material and the finished product could serve as generalized method of standardization and quality control of Ksharsootra.