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An Experimental Study to Standardize the Preparation of Bhandira Guda Varti

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ABSTRACT
Pain is considered as a subjective feeling. It's a noxious stimulus but play an important role in the protection of tissues and allows the body to heal properly along with avoiding a similar damage in the future. From the view point of a surgeon, pain is a challenge, especially in managing the post operative conditions. Many medicines are available for the management of pain like opioids and NSAIDs, but all are having their own complications. In the quest of finding side effect less and long usable medicines for the management of pain, all attention is towards the herbal remedies. There are many medicines in Ayurveda and in folklore use in India which claim analgesic effects. One among this is Bhandira (Clerodendrum infortunatum Linn.). Many experimental studies state that it is having good analgesic effect. This study is attempted to standardize the preparation of Guda Varti (Anal suppositories) from the leaf extract of Bhandira (Clerodendrum infortunatum Linn.) to use it in the post haemorrhoidectomy pain management as a local analgesic agent.
Keywords: *Pain, Haemorrhoidectomy, Herbal Analgesic, Guda Varti*

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INTRODUCTION

Haemorrhoid is considered as disease which occurs in human beings due to various reasons and leads to caudal displacement of the anal cushions and mucosal trauma [1]. It is estimated that around 75% of population in India are having haemorrhoid related issues [2]. The treatment of haemorrhoid depends on its severity, in first and second degree only conservative management is enough, but in third and fourth degree surgical interventions are advocated [3]. In surgical cases the post operative pain is considered as the greatest challenge for the surgeons and the patients. In modern medicines NSAIDs are the primary drug of choice to mitigate pain, but its impact on gastric mucosa and occurrence of gastritis are unavoidable. Opioid drugs may develop dependency and psychological impacts in the patient [4].

Many folklore medicines are there in practice in Indian subcontinent which are very effective in managing pain, but their proper studies are not been conducted. *Bhandira* (*Clerodendrum infortunatum* Linn.) is such a medicinal plant. Some previous studies shows that the saponin extract of this plant leaves are having analgesic effect [5,6]. Here, our effort is to find out a proper standardized methodology to prepare *Guda Varti* (anal suppository) out of the extract from its leaf, which helps in managing post haemorrhoidectomy pain.

MATERIAL AND METHODS

There are two objectives in this study 1) To find out a proper method of extraction so that maximum amount of saponin can be acquired from *Bhandira* leaves. 2) To standardize preparation method of anal suppository from *Bhandira* saponin extract.

Extraction method

For this purpose the fresh leaves of *Bhandira* (*Clerodendrum infortunatum* Linn.) were collected from Alappuzha district, Kerala state. Identification and authentication of the drug done at Care Kerala, Kochin. Shade dried the leaves and pulverised it into powder form using 18 number sieves. Extraction procedure

and Quantification of saponin content were done at Phyto chemistry Lab., at Jawaharlal Nehru Tropical Botanical Garden, Thiruvananthapuram. Three different solvents were used for the extraction purpose.

Alcoholic extraction

Soxhlet method is used for this purpose. 200gm of leaf powder is taken and 3 liters of Petroleum Ether is used under 50°C for a period of 12 hours (20 siphening occurred during the pro cedure)to do the dewashing of fat from the sample. Then the sample is taken out and dried properly. Again soxhlet apparatus is charged with the sample and 3 liters of Ethanol as solvent. Run the procedure at 100oC for a period of 20 hours. Solvent removed completely using Rotavapor machine. 33.27 gms of extract gained from this procedure [7].

Hydro alcoholic extraction

Cold extraction (Lab. Maceration) method used in this procedure. 100 gm of sample drug and 1 liter of 70% Ethanol as solvent were taken in a 1.5 liter conical flask and stirring of the content was done with Magnetic stirrer. The stirring continued for 3 hours and the extract with solvent filtered using whatman's filter paper and kept aside. The same procedure repeated twice more using fresh solvent. The whole 3 liters of solvent mixed together and solvent removed using Rotavapor. 21.08 gm of extract obtained [8].

Water extraction

10 gms of sample drug with 100 ml of distilled water taken in a 200 ml conical flask and stirring done using magnetic stirrer for a period of 3 hours. Filtered using Whatman's filter paper and the filtrate is stored in refrigerator. The procedure repeated twice more using fresh solvent; the whole filtrate mixed together and carefully removed the solvent using Rotovapor machine. 1.30 gm extract obtained [9].

Quantification of saponin

To compare the extracts for its Saponin content was done through HPTLC method. For this purpose a small amount of each extracts were taken and mixed with 70% hydro alcoholic solution in a ratio of 1:100. All the samples were mixed thoroughly with the solvent. A 10×10 cm size HPTLC plate with pre coated silica gel (HPTLC plates silica gel 60F 254 manufactured by E. MERCK KGaA) was taken and loaded the samples using automated loading machine. All the three samples were loaded in the plate in two different quantities (2µl and 4µl) with equal intravels. Mobile phase done in 20×10 cm twin through chamber using Hexane- C hloroform- Methanol in 5:4:1 ratio. Then dried in vacuum desiccator and derivatization done using Anisaldehyde and Sulphuric acid reagent. Then it is dried in hot air oven in 115.5oC for 5 minutes. Then the plate was put in visualiser and checked at 254 nm and 366 nm. Analysis done using win CAT software and conclusions were made. Almost similar Saponin concentration were found in alcoholic extract and hydro alcoholic extract, with alcoholic extract showing a bit more. But the water extract showing much lower concentration of Saponin compared to others [10].

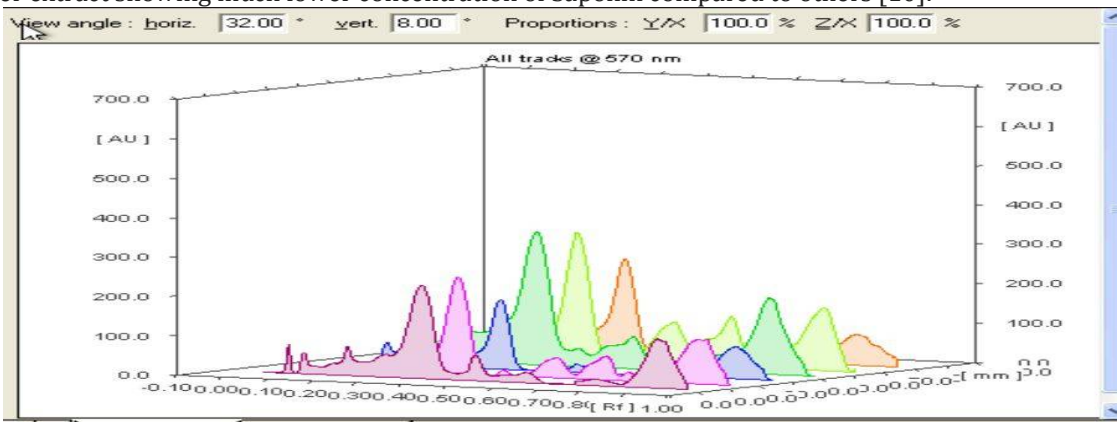

Figure:1 Comparative HPTLC of *Bhandira* leaf extract in different solvent

obal peak table | Export to file

rack	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	1	0.23 Rf	2.9 AU	0.27 Rf	23.2 AU	9.95 %	0.27 Rf	20.8 AU	505.7 AU	4.67 %
1	2	0.27 Rf	20.9 AU	0.34 Rf	210.1 AU	90.05 %	0.40 Rf	0.1 AU	10318.9 AU	95.33 %
2	1	0.24 Rf	15.6 AU	0.24 Rf	21.3 AU	8.62 %	0.26 Rf	0.5 AU	293.3 AU	2.87 %
2	2	0.27 Rf	0.5 AU	0.33 Rf	226.3 AU	91.38 %	0.39 Rf	2.2 AU	9940.3 AU	97.13 %
3	1	0.23 Rf	1.5 AU	0.33 Rf	167.3 AU	100.00 %	0.38 Rf	0.1 AU	6811.6 AU	100.00 %
4	1	0.23 Rf	2.7 AU	0.32 Rf	273.8 AU	100.00 %	0.38 Rf	0.1 AU	14107.0 AU	100.00 %
5	1	0.26 Rf	0.3 AU	0.32 Rf	277.3 AU	100.00 %	0.39 Rf	0.5 AU	13441.7 AU	100.00 %
6	1	0.23 Rf	0.2 AU	0.33 Rf	223.2 AU	100.00 %	0.40 Rf	0.5 AU	10440.4 AU	100.00 %

Figure: 2 Comparison chart of HPTLC study

Preparation of Bhandira guda varti

As the concentration of Saponin was less in water extract, only alcoholic extract and hydro alcoholic extract were used in the *Guda Varti* (anal suppositories) preparations. *Guda Varti* (anal suppositories) from both the extracts were made in three dosage forms like 360mg, 500mg and 1gm. Preparation procedures were carried out at the Quality Control Lab of Parul Institute of Ayurved and Research, Vadodhara, Gujarat. For this we used Coco butter and petroleum wax as base and prepared *Guda Varti*(anal suppositories) with a dimension of 2.6 cm in length and 1.2 cm diameter and weighing 2.83 gms. Coco butter was brought from an authorized vender (Caffrich, Coimbatore, Tamil Nadu, India) and Petroleum wax from Vadodara, Gujarat, India. Coco butter and Petroleum wax were taken in 2:1 ratio and extracts taken in calculated amount for different dosages. First Coco butter and Wax were taken in a glass vessel and heated in 100^{o}C till all the contents liquefies and then the extract added into it and mixed till a homologous mixture obtained. Then this mixture is loaded in the metallic suppository moulds and allowed to cool by itself. After cooling the moulds are opened and the *Guda Varti's* (anal suppositories) are removed, packed in food grade aluminium foils and stored in refrigerator. For the preparation of 12 numbers of 500mg suppositories we used 18.7 gms of Coco butter, 9.4 gms of Wax and 6 gms of extract, similarly each dosages were calculated and prepared *Guda Varti's*(anal suppositories) [11].

Standardization of Bhandira guda varti

Standardization of *Bhandira Guda Varti* (anal suppositories made out of leaf extract of *Clerodendrum infortunatum* Linn.) was done using the methods explained in Remington, The Science and Practices of Pharmacy [11].

RESULTS

Visual Examination

Colour - colour of the product was determined using a colour chart. Shape - Shape of the *Guda Varti*(anal suppositories) was observed and determined. Surface Condition –the finished product was observed for its smoothness, cracks, dark regions, bursts, air bubbles, holes etc. Appearance – appearances like Dry, oily or moist were observed and determined. Feel - by touching the product stickiness hardness were determined. Odour - Odour was determined by smelling the product, a change in odour indicative of a degradation process.

Weight -Ten *Guda Varti's* (anal suppositories) were selected randomly and weighed on an automatic balance. Then its average weight was calculated.

Melting Point - The melting point was determined by placing a small diameter wire into the mould and the *Guda Varti* (anal suppositories) was prepared with the wire in it. After solidification it was immersed in water by holding it on the wire and then temperature of the liquid was raised slowly by 1oC each at every 2 minutes until the suppository slips off the wire. That temperature was noted as the melting point of *Guda Varti*(anal suppositories).

Liquefaction Time - It measures the time required for a suppository to liquefy when subjected to maximum temperature of 37^{o}C.

Physical Stability - Weekly, observed the suppository for signs of discoloration, dryness, cracking, etc for eight weeks and recorded.

Table:1 Analytical Study report of *Bhandira Guda Varti.*

Colour	Dark Green
Shape	Bullet Shape
Surface Condition	Smooth without any cracks
Appearance	Oily
Feel	Hard
Odour	Pungent
Weight	2.83 gm (Average)
Melting Point	36.1oC
Liquefaction Time	5.49 minutes
Physical Stability	No deformities observed for a period of 2 months

DISCUSSION

In this study we tried to standardize the preparation of *Bhandira Guda varti* (anal suppositories made out of leaf extract of *Clerodendrum infortunatum* Linn.), which will help in the post Haemorrhoidectomy pain [1]. We followed all the standard procedures in the preparation of suppositories explained in the pharmacopoeia books. Here we tried different extraction methods of Saponins [2]. While analysing those extracts, against the common belief of saponin having more affinity towards water, we found that the saponin concentration in water extract was low compared to alcoholic or hydro alcoholic extracts. As the saponin from this plant leaves is the key ingredient for its analgesic property we used only the alcoholic

and hydro alcoholic extracts in the preparation. During the preparation of *Guda Varti*(anal suppositories), we tried different doses like 360mg, 500mg and 1gm, in all cases the *Guda Varti* (anal suppositories)shown uniform properties [3-6]. Increasing the concentration of the extract, which is the key ingredient of this preparation, up to 1gm will not affect the stability of the *Guda Varti*(anal suppositories) [7].

Coco butter is a commonly used base for the preparation of the suppositories, so the same was use in this preparation too. But using the Coco butter alone as base posed difficulties during the preparation like delay in solidification and unstable shape of suppositories. To overcome this challenges we used half part of wax along with Coco butter in a 2:1 ratio. The rationale behind adding half part of wax is to maintain structural intactness of the suppository and it won't affect the desired qualities of the suppository like liquefaction time or melting time. Above that the smooth surface, oily surface and hard consistency of the suppository will help in the easy application of it in the anal canal [8-11].

CONCLUSION

As the saponin extract from *Bhandira* (*Clerodentrum infortunatum* Linn) is having analgesic property, this standardized *Guda Varti*(anal suppositories) helps in easy delivering of the drug through anal route. Anal mucosa is having good absorption capacity and the site of tissue damage is at anal region during haemorrhoidectomy, so the action of the drug may be faster. The positive results were observed in our clinical study entitled "A Clinical Study To Evaluate The Effect Of *Bhandira Guda Varti* (Saponin Extract Of *Clerodendrum infortunatum* Linn. Leaf As Suppository) In Post Haemorrhoidectomy Pain Management". More clinical studies are needed to prove the efficacy of this drug and if it is proven effective then it will be a great boon in the management of pain through herb with less or no side effects.

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