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# Preliminary Evaluation of *Eulophia herbacea* tubers mucilage as Gelling Agent

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# Abstract

The mucilage from the tubers of *Eulophia herbacea* (Family orchidacea) was extracted by dissolving in water and precipitating in 90% alcohol, yield (9-11%) of mucilage. Such mucilage when mixed with water, a protective and soothing preparation results. The objective of the present work is to study the *Eulophia herbacea* mucilage as gelling agent. To study the gelling properties, gels were prepared using Diclofenac sodium as model drug. Six batches of drug loaded gels with concentration of mucilage corresponding to 2.5,3.0,3.5,4.0,4.5 and 5% w/w were formulated by using glycerin as wetting agent and thiomersol as preservative. The prepared gels were evaluated for Diclofenac sodium content, pH, rheological studies such as viscosity and extrudability, Consistency, Homogeneity, Spreadability, in vitro diffusion profile and stability studies. The gel prepared with 4.0% of *Eulophia herbacea* tubers mucilage showed desired gel characteristics with better drug release profile when compared with marketed formulation. Stability study revealed that the gel formulations were physically stable.

Key words: Eulophia herbacea tubers mucilage, Diclofenac sodium, gelling agent, Spreadability

#### Introduction

In recent years, plant gums have evoked tremendous interest due to their diverse pharmaceutical applications such as diluent, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository<sup>1</sup>. The prospects of natural polymers are brighter but even here extensive testing will be required. The synthetic polymers have certain such as high cost, toxicity, disadvantages environmental pollution during synthesis, nonrenewable sources, side effects, less patient compliance, etc<sup>2</sup>. While the advantages of natural plant based materials include low cost, natural origin, free from side effects, bioacceptable, renewable source, environmental-friendly processing, local availability (especially in developing countries), better patient tolerance as well as public acceptance, from edible sources,  $etc^3$ .

*Eulophia herbacea* tubers Mucilage (EHM) is available locally and has not been explored as pharmaceutical excipients. Mucilages are polysaccharide complexes formed from sugar and uronic acid units. Mucilages form slimy masses in water, are typically heterogeneous in composition.

Upon hydrolysis, arabinose, galactose, glucose, mannose, xylose and various uronic acids are the most frequently observed components. Mucilages are obtained from rhizomes, seeds especially from tubers. Some are obtained from marine algae, and from selected microorganisms<sup>4</sup>. In present study the tubers of *Eulophia herbacea* tubers were selected for the isolation of mucilage.

*Eulophia herbacea* tubers also known as Kokad-kand in Marathi contains a high proportion of mucilage and it also being used for different therapeutic purposes<sup>5</sup>. The plant has been used medicinally in treatment of tumors of scrofulous glands of neck, worms and rheumatism<sup>6</sup>. When this mucilage is mixed with water it acts as a protective and soothing preparation, which can be applied externally. Hence, the main aim of present work was to evaluate the suitability of *Eulophia herbacea* tubers mucilage as a gelling agent in topical formulation. Diclofenac sodium used as modal drug.

# Materials

The fresh *Eulophia herbacea* tubers were collected from plants growing in hilly region of North Maharashtra, India. The plant was authenticated at the Botany Department of M. J. College, Jalgaon, India. The materials used were Diclofenac sodium was obtained as gift sample from Haffkin Ajanta Pharmaceutical Ltd., Jalgaon. All other chemicals used were of analytical grade and double distilled water was used throughout the experiments.

# **Extraction of mucilage**

The fresh *Eulophia herbacea* tubers were collected and washed with water. The tubers were crushed and soaked in water for 5–6 hours, boiled for 30 minutes and left to stand for 1 hour to allow complete release of the mucilage into the water. The mucilage was extracted using a multi-layer muslin cloth bag to remove the marc from the solution. Acetone (in the volumes of three times to the volume of filtrate) was added to precipitate the mucilage. The mucilage was separated, dried in an oven at 40°C, collected, ground, passed through a # 80 sieve and stored in desiccator at 30°C & 45% relative humidity till use <sup>7-</sup>

#### **Purification of the Mucilage**

The crude mucilage (1 %) was homogenized (Potter homogenizer) with cold dilute trichloro acetic acid solution (5%). The solution was centrifuged (3500 rpm for 20 min), neutralized with sodium hydroxide by drop wise addition and then dialyzed for 30 hours against distilled water. The mucilage was precipitated with ethanol (in the quantities of three times the volumes) and washed successively with ethanol, acetone and diethyl ether. The mucilage so obtained was dried under vacuum (less than 1 Torr at 25°C for 12 hour). The so obtained mucilage was passed through a # 80 sieve and stored in desiccator at 30°C & 45% relative humidity till use<sup>10, 11</sup>.

## Physicochemical properties of mucilage

The physicochemical properties such as solubility, ash values, pre-compression parameters, microbial load, swelling index, loss on drying were determined according to Indian Pharmacopoeial Procedures. The pH of the mucilage was determined using a digital pH meter 962 - P (Max Ltd., India). Table 1.

# Determination of gel forming concentration of mucilage<sup>12</sup>

Gel forming concentration of *Eulophia herbacea* tubers mucilage was determined by mixing the required quantity of mucilage with distilled water by using laboratory stirrer (Remi Motors, India) and allowed to stand for 1 hr, so as to enable the gum to

swell. Thus, base on consistency gelling concentrations of *Eulophia herbacea tubers* mucilage were found out.

#### Preparation of gels<sup>12</sup>

Six batches of gels were prepared corresponding to 2.5,3.0,3.5,4.0,4.5 and 5.0 % w/w Eulophia herbacea tubers mucilage, Diclofenac sodium (1%w/w) as modal drug, glycerin (4%v/w) as wetting agent and thiomerosal (0.01%) as preservative. First the weighed amount of mucilage was wetted by glycerin and half of the quantity of water was then added. The dispersion was allowed to keep in room temperature for 1 hr to obtain the hydrate viscous mixture. The Diclofenac sodium and thiomerosal were dissolved in the rest of water and dispersed slowly to the gel formulations by mixing gently to obtain homogenous gel at room temperature. The gels were obtained after final weight adjusted with water to 100 g and allowed to stand for complete hydration at room temperature for 24 hrs and stored in cool place prior to its use. The composition of gels is mentioned in Table2.

#### **Evaluation parameters**

The prepared gel were evaluated for parameters such as Diclofenac sodium content, pH measurements, Rheological studies such as viscosity and extrudability, in vitro diffusion profile and stability studies.

# Drug content studies<sup>13</sup>

Diclofenac sodium gels (0.5g) were weighed and diluted with about 50 ml of pH 7.2-phosphate buffer in a volumetric flask and appropriate dilutions were made with the same buffer solution. The resulting solution was then filtered using 0.45-mm cellulose acetate membrane filters and the amount of Diclofenac sodium was determined at 276 nm using UV spectrophotometer (Shimadzu UV 1800, Japan). The observations are shown in Table 3.

#### Measurements of pH

The pH was measured in each gel, using a digital pH meter 962 - P (Max Ltd. India), which was calibrated before use. The measurements of pH of each data were in triplicate and the average values are given in Table 3.

# Consistency<sup>14</sup>

The measurement of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fix distance of 10cm in such way that it should fall on the centre of the glass cup filled with the gel. The penetration by the cone was measured from the surface of the gel to the tip of the cone inside the gel. The distance traveled by cone was noted down after 10sec<sup>•</sup> (Table no. 4)

# Homogeneity<sup>14</sup>

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates. (Table no. 4)

# Spreadability<sup>14</sup>

It was determined by wooden block and glass slide apparatus. Weights about 20g were added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slides. Spreadability was then calculated by using the formula, (Table no. 4)

S = M.L/TWhere,

S = Spreadability

M = Weight tide to upper slide

L = Length of glass slide

T = Time taken to separate the slide completely from each other.

#### **Rheological studies**

#### Viscosity

A Brookfield digital viscometer LVDE model with spindle no and speed was used to measure the viscosity in cps of the suspensions. The sample temperature was controlled at  $25\pm1^{\circ}$ c before the each measurement. The results are given in Table 3. **Extrudability**<sup>15</sup>

A simple method was adopted for determination of extrudability in terms of weight in grams required to

extrude a 0.5 cm ribbon of gel in 10 sec from the collapsible tube. The results are given in Table 3.

# In vitro diffusion profile<sup>12</sup>

In vitro diffusion profile of the gel was determined by using Franz diffusion cells. A piece of 47 mm thickness Nylon 6,6 membrane with 0.2 µm pore size used as a barrier. The diffusion studies were carried out at 37±0.5 c using pH 7.2 phosphate buffer as (receptor phase) diffusion medium. One gram of the gel of each formulation was exposed to diffusion study and samples withdrawn at different time intervals such as 0.5,1,2,3,4,5,6 and 8 h with additive of appropriate amount of same fresh buffer solution to keep the volume constant. The samples were analyzed by UV spectrophotometer (Shimadzu UV 1800, Japan) at 276 nm. Cumulative percentage drug release at different time intervals was calculated using the regression equation generated from the standard curve. (Table 3 and Figure 1). Each data point represented the average of three determinations. In vitro release studies were observed for an eighthour period. Market sample of gel was also tested for dissolution profiles and compared with that of prepared gels.

# Stability studies<sup>16</sup>

Prepared gels were stored in glass containers (well stoppered) for three months in the dark at room temperature  $(25\pm1^{\circ}C)$ . They were checked after preparation and monthly throughout three-month period. Physical evaluation of stability of the prepared gel formulation was carried out by visual inspection and rheological tests.

Sr. No.	Tests	Observations
1.	Melting range	Decomposes above 2000 <sup>0</sup> c
2.	PH (1%w/v)	Neutral.
3.	Loss on drying	6 %
4.	Ash value	4.7 %
5.	Acid insoluble ash.	0.3 %
6.	Swelling index	16
7.	Carr index (%)	15.27
8.	Bulk density	0.61 gm/cc
9.	Tapped density	0.72 gm/cc
10.	Hausner ratio	1.18
11.	Angle of repose	$25^{0}$

Table1: Result for physicochemical evaluation parameter of the Eulophia herbacea Mucilage

## Table 2: Compositions of Diclofenac sodium gel

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		Batch Name				
Ingredients	<b>EHM 1</b>	<b>EHM 2</b>	EHM 3	EHM 4	<b>EHM 5</b>	EHM 6
	(%W/W)	(%W/W)	(%W/W)	(%W/W)	(%W/W)	(%W/W)
Eulophia herbacea Mucilage (EHM)	2.5	3.0	3.5	4.0	4.5	5.0
Diclofenac sodium	1	1	1	1	1	1
Thiomerosal	0.01	0.01	0.01	0.01	0.01	0.01
Glycerin	4	4	4	4	4	4
Water	100	100	100	100	100	100

Table 3: Physical characteristics of Diclofenac sodium gels

Batch Name	Drug content % ±S.D.	pH.	Extrudability	Viscosity (cps)±S.D.	Amount of drug release after 8 hr (%)±S.D.
EHM 1	93.13±0.62	7.01	+	1121±3.02	70.58±0.45
<b>EHM 2</b>	93.31±0.24	7.03	++	$1203 \pm 3.45$	74.26±0.31
EHM 3	94.75±0.30	6.98	++	1290±3.69	77.16±0.20
<b>EHM 4</b>	97.92±0.37	7.08	+++	$1402 \pm 3.93$	86.10±0.19
<b>EHM 5</b>	95.16±0.19	7.10	++	$1512 \pm 4.19$	80.82±0.57
EHM 6	94.23±0.42	7.15	++	$1605 \pm 4.57$	75.92±0.83
MP	98.11±0.59	7.12	+++	1521±3.55	86.69±0.18

**Note:** 1) +: good; + +: very good; + + +: excellent. 2) All measurements were made in triplicate. 3) M.P-Market product

Table 4: Physical characteristics of Diclofenac sodium gels

Batch Name	Spreadability (g.cm/sec)	Consistency (60 sec)	Homogeneity
EHM 1	6.2	6.0	Homogeneous
<b>EHM 2</b>	6.2	6.0	Homogeneous
EHM 3	6.0	7.0	Homogeneous
EHM 4	6.6	7.0	Homogeneous
EHM 5	6.5	7.0	Homogeneous
EHM 6	6.8	7.0	Homogeneous
MP	6.5	8.0	Homogeneous



Fig no. 1: In vitro dissolution profile of prepared gels.

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