



Formulation and Evaluation of Herbal Eye Gel from *Heliotropium indicum* linn leaf extract for Conjunctivitis

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ABSTRACT

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years. The present work was designed with the aim to formulate and evaluate the ocular herbal gel containing *Heliotropium indicum* linn leaf extract. Cold infusion of the leaves used to remove cataract in the eye. The ocular herbal gel formulation was prepared from aqueous extract in varied concentration and was evaluated. The gel was formulated by using carbopol 934, EDTA, Benzalkonium chloride, and required amount of distilled water. Then the ocular pH 7.4 was

maintained by drop wise addition of triethanolamine. The herbal ocular gels were sterilized and assessed for various parameters like clarity, pH, physical appearance, physical stability, viscosity, uniformity of drug content, spreadability and anti- microbial studies. Stability studies were carried out as per ICH guidelines for 6 months at different temperatures and humidity. The formulations in gel were found to be more stable at ambient, refrigerator and incubated temperature. The results showed that formulation g1GL7 consisting aqueous leaf extract of *Heliotropium indicum* linn have better stability than other formulations.

KEYWORDS:

Heliotropium indicum linn, Ocular herbal gel, Bacterial conjunctivitis and Anti- microbial.

1. INTRODUCTION

In improving the quality of human life plants have played a significant role. Herbal medicine is based on the principle that plants contain constituents that can promote health and alleviate illness. All over the world the plants research has increased and the collected evidences showed the immense potential of medicinal plants used in various traditional system. There are many medicinal plants generally used in ocular diseases which are easily available and possess biological activity. The efficacy of many traditional medicines in curing ocular disorders is recognized by modern science also. In pink eyes there is an inflammation of conjunctiva which is also known as Conjunctivitis which is the most common disorder encountered in ophthalmology. It causes redness, burning sensation.

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sensitivity to light, dryness, grittiness sensation, itchy and scratchy feeling, watering of eyes, and swelling of eyelid. The conjunctivitis caused due to bacteria known as bacterial conjunctivitis.

Heliotropium indicum(HI) linn (Family Boraginaceae) is a medicinal plant. It has various medicinal uses in the treatment of disease conditions such as abdominal pains, amenorrhoea, aysmenorrhoea, skin rashes, wounds, hypertension, ocular infections, convulsion and dizziness. Cold infusion of the leaves used as an enema stops abdominal pains; this preparation also removes cataract in the eye; the juice from the leaves is squeezed into the eye to stop dizziness; decoction of the whole plant is used to treat convulsion in children. Other medicinal uses of *Heliotropium indicum* linn comprises the use of juice of the leaves as an antiseptic and anti- inflammatory agent when applied to wounds, sores, boils, gum boils and pimples on the face. Boiled with castor oil, it is applied to sores from scorpion bites and also locally used in treating ophthalmic disorders like erysipelas and pharyngodynia^{1,2}. Our aim of the

project is to prepare and evaluate the eye gel from HI leaf extract to treat bacterial conjunctivitis.

2. MATERIAL AND METHODS

Collection and preparation of plant powder

The leaves of *H. indicum* were collected in the month of December from Nagercoil, TN and dried in shade. The plant was authenticated by Dr. D. Stephen, Madurai medical college, TamilNadu. The shade dried leaves were powdered to get a coarse powder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

Preparation of extracts

The leaf of the plant were dried in shade for about 3 weeks and ground by using a mixer to a coarse powder. Powders of leaf were first extracted with the petroleum ether for defatting and then successively re-extracted with ethyl acetate and 70% acetone for 48 hrs. Obtained acetone extract was filtered and dried by using rotary flash evaporator. The 20gm of coarse powder of leaf was boiled in 400 ml of distilled water and were further heated at 60–70°C to a concentrated solution (~50 ml). Extracts were subsequently filtered through 0.22µm filters and concentrated to dry mass by using vacuum distillation. The percentage yield was calculated. The aqueous extracts were used for further study.

Materials

Carbopol 934 was purchased from Rolex chemical Industries, Mumbai, Petroleum ether, Ethyl acetate, Acetone, EDTA, BKC, and triethanolamine used were of analytical grade.

Selection of vehicle

The solubility of the extracts was checked in various solvent like ethyl acetate and 70% acetone and distilled water. (table:1)

Incompatibility studies by FT- IR

The crude drug and excipient compatibility study was determined by FT-IR (Fourier Transformer Infrared Spectroscopy) using KBR pellets of 0.1 mm. The IR spectra of the extracts are compared with IR spectrum of combination of extracts and all the excipient to check the interaction. (Figures:1-3)

Preparation of ocular herbal gel³

Specified quantity of carbopol in STF (STF: simulated tear fluid- pH 7.4)for 24hrs was soaked. The solution was stirred with an overhead stirrer, EDTA solution was added while stirring. After 24 hrs the mixture was mixed it properly, then the dissolved and filtered drug (HI Extract) solution(1gm in 100ml) was added to the above solution mixture and triethanolamine was added drop wise to the formulation for adjustment of required ocular pH (7.4) and to obtain the gel at

required consistency. At last 2 drops of 0.02% BKC was mixed it well and the gel was sterilized at 121°C for 20mins in autoclave and uses it for further studies.

Table: 1 Formulation chart for HI leaf aqueous extract of GL1-GL9 formulations

Ingredients	Ingredient concentration (% w/v)								
	GL1	GL 2	GL3	GL 4	GL 5	GL 6	GL 7	GL8	GL9
HI leaf aqueous extract	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
BKC	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol 934	0.4	0.6	0.8	1	1.2	1.4	1.6	1.8	2
Tri ethanol amine	2	2	2	2	2	2	2	2	2
pH7.4 Buffer (ml)	100	100	100	100	100	100	100	100	100

EVALUATION OF OCULAR HERBAL GEL FORMULATIONS

Physical evaluation³

Visual Appearance and Clarity

Clarity and transparency are the most important characteristic features of ophthalmic preparations. The formulations were examined for visual appearance, clarity and transparency by visual observation against a white and black background to check the presence of any particulate matter. Results shown in table:3

Measurement of pH

The preparation should be non-irritating as it is to be instilled to the eye. To ensure that the preparation has same pH as that of lachrymal fluid, the pH of the formulation after addition of all the ingredients was measured using digital pH meter. Results shown in table:3

Spreadability^{4,5}

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 kg weighted was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 g. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability. (Table: 3) Spreadability was calculated using the following formula: $S = M \times L / T$

Where, S = Spreadability,

M = Weight in the pan (tied to the upper slide),

L = Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other

Homogeneity

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: 3)

Rheological studies^{6,7}

The rheological behavior of the formulations was investigated as a function of pH. Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. Viscosity is an important feature to determine the resistance of flow of gel formulation so that it can spread on the eye properly at pH 7.4 with high viscosity. The formulations exhibited pseudo plastic rheology. Rheological studies of the prepared formulations were carried out by Brookfield DV-II+ viscometer using LV-2 spindle. The viscosities of the formulations were determined at different speed conditions (5, 6, 10, 12, 20, 30, 50 rpm)(Fig: 4).

Antibacterial activity by agar well diffusion method^{8,9}

In this method a previously liquefied medium was inoculated with 0.2 ml of *Staphylococcus aureus* suspension having a uniform turbidity at temperature of 40° C. 20 ml of culture medium was poured into the sterile petri dish having a internal diameter of 8.5 cm. Care was taken for the uniform thickness of the layer of medium in different plates. After complete solidification of liquefied inoculated medium, the wells were made aseptically with cork borer having 6mm diameter. In each of these plates gel was placed carefully. Plates were kept for pre diffusion for 30 minutes. After it normalized to room temperature, the plates were incubated at 37° C for 24 hours in case of bacteria. After incubation period was over, the zone of inhibition was measured with help of Hi-media. The inhibitory effect of gel formulations were compared with marketed ciprofloxacin eye gel for bacteria. Results shown in table:3

3. RESULTS AND DISCUSSION

Selection of vehicle

Table: 2 Solubility data for leaf extract of *Heliotropiumindicum* Linn

S. No	Plant Part	Extract / solvent	Solubility data (mg/ml)
1.	Leaves of <i>Heliotropiumindicum</i>	Aqueous	485
2.		Ethyl acetate	324
3.		70% acetone	298

The result shown in (table:1) solubility data depicts that the aqueous solubility is more for the selected parts of the plant. So the further studies were carried out with aqueous extract of leaf of HI for the formulation of eye gel.

Incompatibility studies by FT- IR

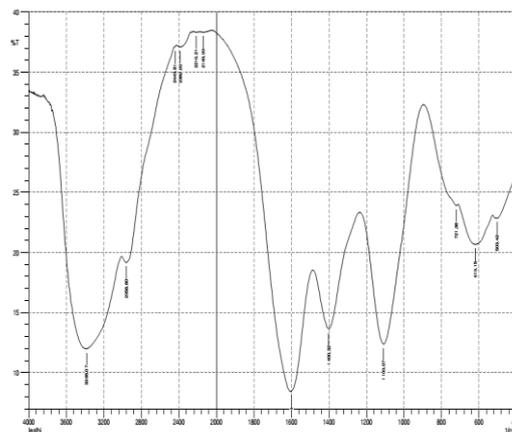


Figure: 1 FT- IR spectra for leaf aqueous extract

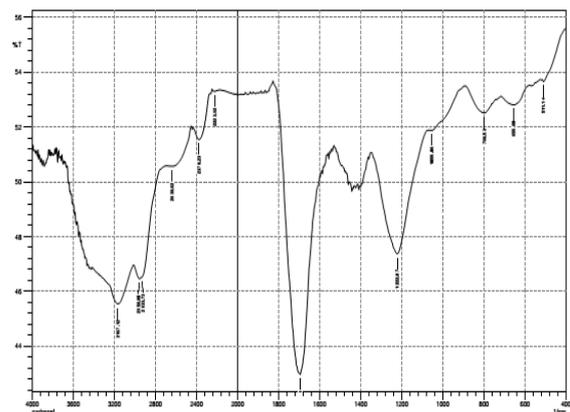


Fig: 2 FT- IR spectrum for Carbopol Preliminary Evaluation

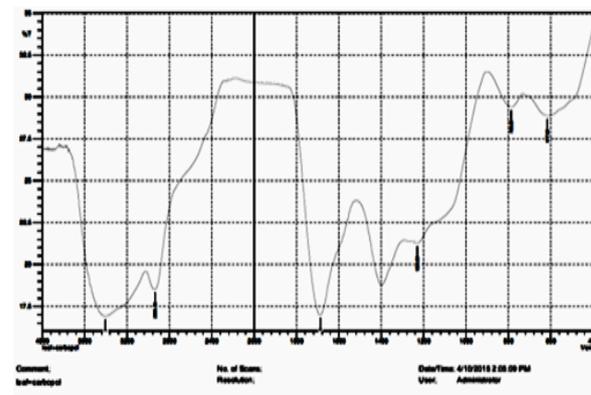


Figure: 3 FT- IR spectrum for Carbopol+leaf extract

The FT-IR spectrum of pure Carbopol934 were 2958, 1712, 1222, 1055, 799 cm⁻¹ wave number as

major peaks. Mixture of the aqueous extract of leaf of HI and carbopol showed no considerable changes in the IR peaks.

Preliminary evaluations

Formulation Code	Colour	Visual appearance	Clarity	Spreadability	pH
				(g.cm/sec)	
GL 1	Slight brownish	Translucent	Clear	12.65	7.3
GL 2	Slight brownish	Translucent	Clear	13.57	7.3
GL 3	Slight brownish	Translucent	Clear	14.28	7.4
GL 4	Slight brownish	Translucent	Clear	15.24	7.3
GL 5	Slight brownish	Translucent	Clear	17.25	7.4
GL 6	Slight brownish	Translucent	Clear	19.58	7.3
GL 7	Slight brownish	Translucent	Clear	21.68	7.4
GL 8	Slight brownish	opaque	opaque	24.58	7.4
GL 9	Slight brownish	opaque	opaque	26.74	7.3

Table: 3 Evaluation of HI leaf aqueous extract of GL1-GL9 formulations

These preliminary evaluations like appearance was done by observing the formulation against white background, clarity, were evaluated by visual observation. All batches of gels were clear and the pH of the formulation was adjusted 7.3 to 7.4 to avoid the ocular irritation. Clarity of all formulations was found to be satisfactory. The pH was within acceptable range and hence would not cause any irritation upon administration of the formulation. The values of spreadability indicate that the gel is easily spreadable by small amount of shear. Spreadability of formulation GL7 was found to be 21.68gcm/sec. Hence spreadability of GL7 formulation was good as compared to other formulations.

Rheological studies

The rheological studies at pH 7.4, the gel formulations were shown high viscosity. Buffers play a pivotal role in formulating ophthalmic gels.

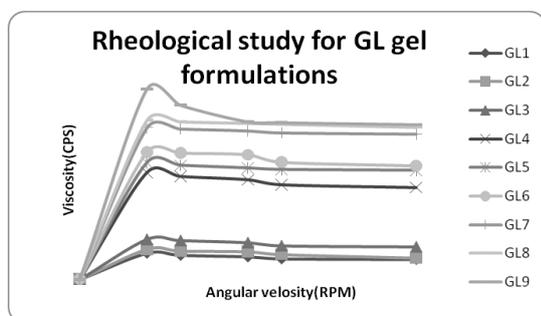


Figure: 4 Rheological studies for GL1 – GL9 herbal gel formulations

They contribute significantly to chemical stability and clinical response and also influence the comfort and safety of the product. Carbopol polymer forms stiff gel when the pH is raised. Autoclaving of polymeric solutions and terminal sterilization by UV irradiation had no effect on the pH and viscosity of the formulations.

The order of viscosity of all formulations was GL9, (carbopol -2%) > GL8 (carbopol -1.8%) > GL7 (carbopol -1.6%) > GL6 (carbopol -1.4%) > GL5 (carbopol -1.2%) > GL4 (carbopol -1%) > GL3 (carbopol -0.8%) > GL2 (carbopol -0.6%) > GL1 (carbopol -0.4%). Among all GL7 (925.4 CPS) was selected because they exists optimum viscosity.

Formulations were shear thinning and an increase in shear stress was observed with increase in angular velocity (pseudo-plastic rheology) (Fig:4) at pH7.4 (the pH of the tear fluid) gels shown high viscosity. The administration of ophthalmic preparations should influence as little as possible the pseudo-plastic character of the precorneal tear film. Since the ocular shear rate is very high, ranging from 0.03 s⁻¹ during interblinking periods to 4250–28,500 s⁻¹ during blinking viscoelastic fluids with a viscosity that is high under low shear rate conditions and low under the high shear rate conditions are often preferred.

Antibacterial activity by agar well diffusion method

Table: 4 Zone of inhibition for the formulation GL7

Formulation code	zone of inhibition(mm, mean ± SD, n=3)
Standard	20±0.05
GL7	13±0.02

The antimicrobial efficacy study was performed for selected gel formulation GL7 showed antibacterial activity against *Staphylococcus aureus* with a zone of inhibition of 13 ± 0.02 mm. The study indicated the formulation GL7 retained its antimicrobial activity when formulated as gel forming ophthalmic system against selected *S. aureus*.

4. CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. It is a very good attempt has made to establish the herbal gel containing aqueous leaf extract of *Heliotropium indicum* Linn. The antibacterial studies revealed that the developed herbal formulation GL7 consisting aqueous leaf extract of *Heliotropium indicum* Linn comparatively better than other formulations to treat bacterial conjunctivitis. The gel also shows the good antimicrobial activity against the microbes used. So the developed herbal formulation is a viable alternative to conventional synthetic eye gel.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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