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Formulation and Evaluation of Herbal Emulgel Loaded with Extract of *Cedrus deodara* for its *In-Vitro* Anti-Inflammatory Activity

Research Article

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Abstract

Ayurveda is the base of Indian Systems of Medicine. The researchers are greatly interested in discovering the potential of natural bioactive components. Remedial effects are produced by different plant extracts. *Cedrus deodara* is found in North India, Pakistan, Afghanistan, Tibet and Nepal. It contains deodarin, cedodarin, taxifolin, myrcene, iso-pimpillin, α-pinene, β-pinene, cedrin, deodarone, sesquiterpene, cedrinoside, dihydromyricetin, matairesinol, atlantone. It is used in treatment of inflammation, ulcer, pain, hyperglycemia, infection, insomnia, skin disorder, blood disorder, apoptosis, fever. Recently in-vivo and in-vitro studies have indicated its anti-inflammatory and analgesic property. The objectives of proposed research work are to formulate and evaluate the herbal emulgel loaded with extract of *Cedrus deodara* and evaluate for its In-Vitro Anti-Inflammatory Activity. *Cedrus deodara* heartwood was extracted using hydro-alcoholic solvent. Phytochemical evaluation of extract was done. Six batches of herbal emulgel were formulated. The prepared herbal emulgel was evaluated for pH, spreadability, viscosity, consistency, appearance, color, washability and appearance. In-vitro anti-inflammatory activity of extract and formulation were done using HRBC Membrane stabilization assay and protein denaturation assay. The proposed research work concludes that the newly formulated herbal emulgel loaded with extract of *Cedrus deodara* can be used in future for anti-inflammatory effect.

Key Words: *Cedrus deodara,* Herbal Emulgel, Anti-inflammatory Activity, HRBC Membrane, Deodarin.

Introduction

Humans have been using medicinal plants in the treatment of disease since early ages. They knew the importance of medicinal plants in drug development. Different phytoconstituents present in plants are responsible for various therapeutic effects. Almost 45000 plant species are present in different region of India, hence India is often called the emporium of medicinal plants. Wild sources provide supply of medicinal plants to industry. Novel medicines can be developed by taking in consideration traditional folk treatment. Primary health care needs of people are mostly fulfilled by the phytoconstituents (1).Plant constituents have main role in curing illness. Plant based drugs are inexpensive compared to modern synthetic medicines. Different parts of plants are exploited for treating various disease (2).There are large number of patients going for herbal therapy. Herbal medicines have wide range of benefits. Herbal medicines have good compatibility with human body. They are cheaper in cost. They have comparatively less side effects. Inflammation can be cured by using plant and plant extracts. *Curcuma longa* Linn, *Zingiber officinalis* Roscoe, *Borago officinalis* Linn, *Oenothera biennis* Linn (Evening primrose), *Harpagophytum procumbens (Devil's claw)*and *Cedrus deodara* Roxb are traditionally reported anti-inflammatory plants (3).

Cedrus deodara is found in North India, Pakistan, Afghanistan, Tibet and Nepal. It contains deodarin, cedodarin, taxifolin, myrcene, iso-pimpillin, α-pinene, β-pinene, cedrin, deodarone, sesquiterpene, cedrinoside, dihydromyricetin, matairesinol, atlantone. It is used in treatment of inflammation, ulcer, pain, hyperglycemia, infection, insomnia, skin disorder, blood disorder, apoptosis, fever. Recently in-vivo and in-vitro studies have indicated its anti-inflammatory and analgesic property (4).

The emulgel are type of formulations mainly used for topical delivery. When emulsion is incorporated into gel, it is called as emulgel shows dual control release property and it is the recent topical NDDS technology. Emulgel is used to treat pains and aches which are in the form of muscle ache, back ache and arthritis (5).The objectives of proposed research work are to formulate and evaluate the herbal emulgel loaded with extract of *Cedrus deodara* and evaluate for its In-Vitro Anti-Inflammatory Activity.

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Material and Methods

Collection and Authentication of plant materials

The raw material *Cedrus deodara* Heart wood was procured from KLE Ayurveda pharmacy. It was authenticated from SHRI B.M.K Ayurveda Mahavidyalaya, Belagavi.

Processing of the Plant Material

After authentication, *Cedrus deodara* was dried and was powdered. This powder was further used to obtain the extract.

Extraction of plant material

The powder of *Cedrus deodara* was macerated with 70% alcohol for 48 hours. The marc was extracted with 70% ethanol by using Soxhlet apparatus. Both the extracts obtained from maceration and soxhlation were combined. This combined extracts was further concentrated.

Preliminary Phytochemical Screening

Phytochemical screening is performed to identify presence of different Phytoconstituents. The extract was dissolved in ethanol. It was filtered and filtrate was used to carry out test for alkaloids, flavonoids, carbohydrates, saponins, tannins, steroids and terpenoids.

Formulation of Emulgel

Different batches of emulgel were prepared by using different gelling agents and by varying their concentration as per the Table 1. The emulsion was prepared using 2 % of *Cedrus deodara* extract. The gelling agents were soaked in water and triethanolamine was added to adjust the pH. The emulsion was added to the gel and stirred vigorously to form emulgel (6).

Table No. 1: Herbal Emulgel Composition of Different Batches (%w/w)

Composition	F1	F2	F3	F4	F5	F6
Cedrus deodara extract	2	2	2	2	2	2
Sodium CMC	2	3	-	-	-	-
Carbopol-940	-	-	1	1.5	-	-
Sodium alginate	-	-	-	-	2	3
Propylene glycol	5	5	5	5	5	5
Ethanol	5	5	5	5	5	5
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben	0.01	0.01	0.01	0.01	0.01	0.01
Sesame oil	7	7	7	7	7	7
Span-20	1	1	1	1	1	1
Tween-80	0.5	0.5	0.5	0.5	0.5	0.5
Triethanolamine	1.2	1.2	1.2	1.2	1.2	1.2
Water	qs	qs	qs	qs	qs	qs

Evaluation of Emulgel

The prepared herbal emulgel was evaluated for pH, spreadability, viscosity, consistency, appearance, color, washability and appearance.

Physical appearance

The Physical appearance includes study of color, homogeneity, consistency, appearance, etc.Color was noted by visual observation. Homogeneity of emulgel was checked by rubbing the emulgel between fingers. The appearance of emulgel was checked by visual observation. Emulgel was applied to skin to check its consistency.

Spreadability

The emulgel was sandwiched between 2 petri plates and the diameter of circle of spreaded emulgel was used to determine the spreadability. 1 gram of emulgel was weighed and placed on a petri plate. Other petri plate was placed on its top and weight of 50 grams was placed on the top of petri plate for about 60 seconds. After completion of 60 seconds the diameter of circles formed from the spreaded emulgel were measured in triplicate. The average of the reading was calculated. The reading was put into the following formula.

$$S = \frac{M \times L}{T}$$

S: Spreadability
M: Mass
L: Diameter
T: Time

Viscosity

The viscosity of emulgel was estimated using Brookfield viscometer. 1 gram of emulgel sample was taken. The spindle was rotated at the speed of 50 rpm. Readings were taken in triplicate and average of readings were calculated (7).

pH

The pH of the formulated emulgel batches were measured using digital pH meter. 0.5 grams was dissolved in 10 ml of distilled water and electrode was dipped in it to measure the pH (8).

Stability studies

The stability studies of emulgel were carried out by keeping the samples at 5 °C, 25 °C/60% RH, 30 °C/ 65% RH, and 40 °C/75% RH for a period of 3 months. The samples were tested at interval of 15 days. They were evaluated for its appearance, pH, viscosity and spreadability (8).

In vitro anti-inflammatory

Human Red Blood Cell (HRBC) Membrane Stabilization Method

The Human Red Blood Cell (HRBC) membrane stabilization method was used to evaluate the anti-inflammatory activity of extract of *Cedrus deodara* heart wood. 2-3ml of blood was collected from healthy individual. The equal quantity of Alsever's solution was

added to blood. iso-saline was added to the above mixture. This mixture was centrifuge for 5 minute to get Human Red Blood Cell (HRBC) suspension. Equal amount of sample was added to HRBC suspension. 100, 200, 300 µg/ml of concentration of sample were prepared. It was incubated at 37^{0}C for 30 minutes. The mixtures were centrifuged for 5 minutes. Alsever's solution and blood were taken as negative control. Aspirin was taken as a standard. The supernatant solution obtained from centrifugation was used to carry out estimation using UV Spectroscopy at wavelength 560 nm.

Inhibition of Protein Denaturation

Around 0.02ml of sample was taken. 0.2 ml of 1% Bovine Albumin was added. 4.78 ml of PBS (Phosphate buffer saline) was added to the mixture. This mixture was mixed and it was incubated for 15 minutes. It was heated for 5 minutes on water bath at 70^{0}C. The mixture was cooled. UV spectrophotometry used to check absorbance at wavelength 660 nm. Control used was phosphate buffer. A was taken as a standard. The % of inhibition of protein denaturation was calculated (9).

Results and Discussion

The heartwood of *Cedrus deodara* was collected and authenticated. The drug was dried and grinded into coarse powder. The powder was macerated for 48 hours using hydro alcoholic solvent. The marc that was obtained was subjected to Soxhlet extraction. The extracts were combined and further concentrated. The extract obtained was used to study the phytochemical and physico-chemical parameters. The presence of alkaloids, tannins, carbohydrate, flavonoids and terpenoidswere determined by performing phytochemical test. The *Cedrus deodara* showed presence of alkaloids, tannins, carbohydrates, flavonoids, and terpenoids (Table 2).

Table No. 2: Phytochemical Screening of *Cedrus deodara*

Phytoconstituent	Test	*Cedrus deodara*
Alkaloids	Dragendroff's test	+
	Mayer's test	+
	Hagers's test	+
	Wagner's test	+
Carbohydrates	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
Flavonoids	Shinoda test	+
	Sulphuric acid test	+
Tannins	5 % Fecl3	+
	Lead acetate	+
Terpenoids	Salkowski's test	+
Saponins	Foam test	+
Steroids	Salkowski's test	+

Emulsion was prepared. Emulgel was prepared using different gelling agents like Carbopol-949, Sodium CMC and Sodium alginate. The concentration of gelling agent was decided on basis of review of literature. 6 formulations of emulgel were formulated. The prepared batches were evaluated for its colour, pH, greasiness, Spreadability, viscosity and appearance.

The formulated batches had beige colour. The formulations F1, F3, F4 and F5 had good consistency whereas F2 and F6 had verygood consistency. The nature of all batches were non-greasy. The washability of all the formulations was good. Viscosity of formulations ranged from 1259-2125. The pH of formulation ranged from 6.29-6.37. Spreadability of formulations were found in range of 5.1-6.2. Stability studies of emulgel were carried per ICH guidelines. Batch F2 was found to be optimized Table 3.

Table No. 3: Evaluation Parameters of Emulgel

Parameters	F1	F2	F3	F4	F5	F6
Color	Beige	Beige	Beige	Beige	Beige	Beige
Consistency	Good	Very good	Good	Good	Good	Very good
Homogeneity	Good	Good	Good	Good	Good	Good
Appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Greasiness	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy
Washability	Washable	Washable	Washable	Washable	Washable	Washable
pH	6.30	6.35	6.29	6.37	6.34	6.32
Spreadability	5.9	5.6	6.2	5.7	5.5	5.1
Viscosity	1701	2125	1259	1582	1830	2110

In vitro Anti-inflammatory Activity

In-vitro anti-inflammatory activity of extract and optimized formulation was accessed. HRBC membrane stabilization method and protein denaturation method were two methods used to access anti-inflammatory activity. The extract showed good anti-inflammatory activity. The formulated emulgel also showed good anti-inflammatory activity (Table4&5, Figure 1 & 2). The protein denaturation by extract were presented in Table 5 &6, Figure 3 &4).

Table No. 4: Prevention of lysis by *Cedrus deodara* extract

Concentration	Absorbance	Prevention of
300	0.160	36.75
200	0.179	29.01
100	0.189	25.29
Aspirin	0.156	38.33
Negative control	0.253	

Table No. 5: Prevention of lysis by emulgel

Concentration (µg/ml)	Absorbance	Prevention of lysis.
300	0.166	34.38
200	0.180	28.85
100	0.195	22.92
Aspirin	0.156	38.33
Negative control	0.253	

Figure 1: Prevention of Lysis by Cedrus deodara Extract

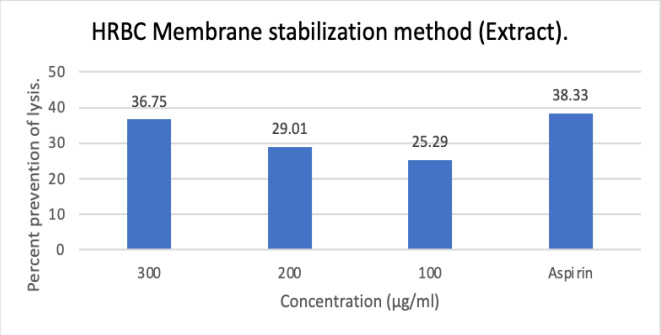


Figure 2: Prevention of Lysis by *Cedrus deodara* Emulgel

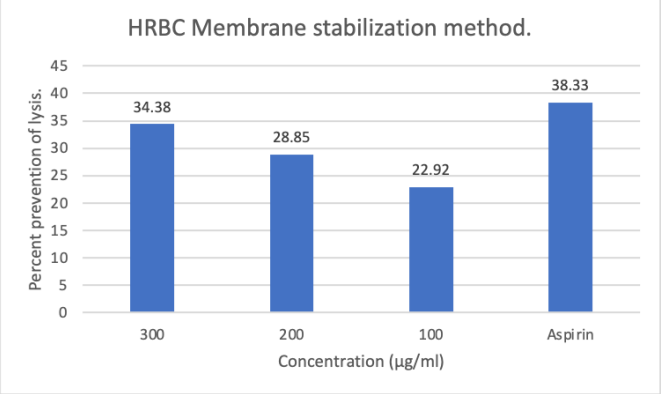


Table No. 5: Protein Denaturation *Cedrus deodara* Extract

Concentration (µg/ml)	Protein denaturation	
	Cedrus deodara	Standard
100	45.31	70.7
200	49.21	75.39
500	53.9	81.25
1000	58.98	86.71

Figure 3: Protein Denaturation by Extract

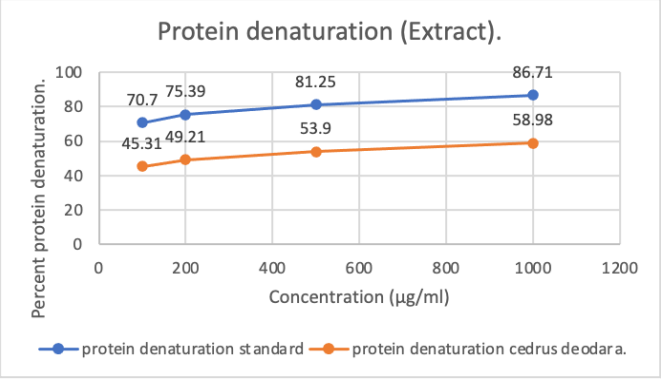
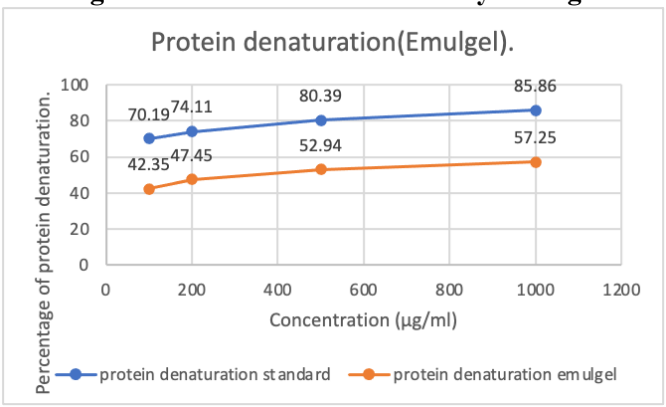


Table No. 6: Protein Denaturation by Emulgel

Concentration (µg/ml)	Protein denaturation	
	Emulgel	Standard
100	42.35	70.19
200	47.45	74.11
500	80.39	80.39
1000	85.86	85.86

Figure 4: Protein Denaturation by Emulgel



Conclusion

Developing and developed countries are using herbal medicines for thousands of years for treatment of various disease. Our findings confirm that the hydroalcoholic extract of *Cedrus deodara* heart wood shows good anti-inflammatory activity. We have formulated emulgel using various gelling agent. The optimized formulation was found to be stable and showed good anti-inflammatory activity. So *Cedrus deodara* emulgel can be used as anti-inflammatory agents for topical drug delivery.

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