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Preparation and evaluation of herbal gel containing *Clerodendrum serratum* for anti-inflammatory activity

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ABSTRACT

The present study was aimed to prepared and characterize gel formulations of alcoholic extract of Clerodendrum serratum (Verbenaceae) using different polymers as gelling agents in two concentrations and also to evaluate anti-inflammatory activity of gel. Gelling agents used in this study, such as carbopol 934, HPMC K 100 M and Xanthan gum were selected for preparation of different gel formulations. The prepared gels were evaluated for physical appearance, pH, viscosity, extrudability, spreadability, anti-inflammatory activity and also skin irritation to observe toxicity or side effects. At last the stability study performed to confirm the stability of final formulation. It was inferred from the results that gel formulations prepared by Xanthan gum found to be best formulation among the prepared batches. The prepared gels were evaluated for anti-inflammatory activity by in-vitro method using Diclofenac Sodium gel as the reference anti-inflammatory gel. And Xanthan gum formulation having root extract in gel shown significant inhibition in in-vitro method.

Key words: Anti-inflammatory, *Clerodendrum serratum*, Protein inhibition, Diclofenac sodium

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INTRODUCTIION

The plants belonging to Verbenaceae family are found to be a rich source of substances of phytochemical constituents. Many plants from this family are used in traditional system of medicines.

Clerodendrum serratum is a small shrub distributed in the deciduous forests of the wetern ghats of India. Different parts of the species *Clerodendrum serratum* are used in Ayurveda and other folk medicines for the treatment of shwasa (breathlessness), kasa (cough), vrana (wound), shotha (sweeling) and many vataja disorders (neurological disorders).[1]

The word inflammation, a defensive reaction to injury with classical signs of warmth, reddening, pain, swelling and loss of function, which is of an acute or chronic type.[2] This inflammation is also observed in cancer[3], bowel syndrome and Alzheimer's diseases. The characteristics of inflammation are humorous like reddening (visible), swelling (odema), soreness (pain) and corresponding histological changes. Non-steroidal and steroidal drugs are generally used to treat inflammation. However, these drugs have side-effects like nausea, vomiting etc.[4]

It is well known that topical gels are more popular among all the topical preparations due to ease of applications. The few studies on topical gel formulations reported the therapeutic effects of drugs in a variety of experimental inflammation and inflammatory pain condition.[5,6]

The present research has been undertaken with the aim to develop a topical gel formulation with alcoholic leaves and root extract, prepared using carbopol 934, Xanthan gum and HPMC. During the development step of formulation it was evaluated stability, spreadability, extrudability and organoleptic characters. Further, the in vitro anti-inflammatory activity of the gel formulation was studied using protein inhibition method.

MATERIALS AND METHODS

Plant collection and authentication

The fresh leaves and roots of *Clerodendurm serratum* (Linn) moon (Verbenaceae) were collected at the flowering stage in August from side Warna River, Yelapur, Tal-Shirala, (Sangli), Maharashtra State, India. It was authenticated and taxonomically identified by Dattajirao Kadam Arts, Science and Commerce College, Ichalkaranji.

Chemicals

Ethanol (Loba Chemie, Pvt Ltd., Mumbai), Carbopol 934 (Corel Pharma), HPMC K 100 M (Research Lab, Mumbai), Xanthan Gum, Methyl paraben, Propyl paraben (Allay Pharma, Mumbai), Propylene Glycol (Loba Chemie, Pvt. Ltd, Mumbai), Tri-ethanolamine (Loba Chemie, Pvt. Ltd).

Preparation of plant extract

In the present study, the leaves and roots were carefully selected wash to remove impurities and shade dried. The dried material was reduced to fine powder in the mechanical grinder. The fine powder was passed through sieve no.43 and stored in an airtight container for further use. About 100 gm of powdered material was extracted with ethanol as a solvent by hot extraction method using Soxhlet apparatus. The extraction was continued until the solvent in the thimble became clear then few drops of solvent were collected in the test tube during the completion of the cycle and chemical test of the solvent was performed.

After each extraction, the extract was evaporated to dryness in rotary evaporator. Moreover, some part of the extract was preserved for preliminary Phytochemical screening for the detection of various plant constituents and rest extract was used for formulation of gel batches.

I. In-vitro anti inflammatory study of extract:

- **Inhibition of albumin denaturation[7,8]**

The anti inflammatory activity of *Clerodendrum Serratum* was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima *et al* and Saket *et al* followed with minor modifications. The reaction mixture was consists of test extract and 1% aqueous solution of albumin fraction, pH of the reaction mixture was adjusted using small amount of 1 N HCL. The sample extract were incubated at 37^0C for 20 min and then heated to 51^0C for 20 min, after cooling the samples the turbidity was measured at 660 nm. The experiment was performed in triplicate. The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition= (abs Control-Abs Sample) × 100/ Abs control

II. Drug-Excipients compatibility study:-

Study of interaction of the drug with excipients by physical compatibility study:-

Each excipients used in the formulations was blended with the drug levels that are realistic with respect to the final dosage form. Each excipient was thoroughly blended with drug extract to increase drug-excipients molecular contacts and also to accelerate the reaction if possible. Each drug extracts excipients blend was taken separately into vials and kept for one month study at 40^0c and at 75% RH for 2 weeks and observe the changes. After 30 days storage of drug extract with excipients in various ratios at room temperature, samples were observed for physical changes but there were no physical changes observed in the mixture of *Clerodendrum Serratum* extract and polymer combination. **(Table no. 1)**

Preparation of topical gels (Table no. 2, 3) [9,10]

Preparation of topical gel of Carbopol 934

Accurately weighed Carbopol 934 was taken in a beaker and dispersed in 50 ml of distilled water. Kept the beaker aside to swell the Carbopol for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Take 5 ml of propylene glycol and required quantity of Extract. Take 5 ml propylene glycol in another beaker and add weighed quantity of propyl paraben and methyl paraben to it and stirred properly. After all

Carbopol dispersed, Extract and preservatives solutions were added with constant stirring. Finally volume made up to 100 ml by adding remaining distilled water and Triethanolamine was added drop wise to the formulations for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency.

Table no.1: Physical Observations of Compatibility Study

Batch	Caking		Discoloration		Liquification	
	Initial	1 Month	Initial	1 Month	Initial	1 Month
Extract	No	No Change	No	No Change	No	No Change
Carbopol 934	No	No Change	No	No Change	No	No Change
Extract + Carbopol 934	No	No Change	No	No Change	No	No Change
HPMC K 100 M	No	No Change	No	No Change	No	No Change
Extract + HPMC K 100 M	No	No Change	No	No Change	No	No Change
Xanthan gum	No	No Change	No	No Change	No	No Change
Extract + Xanthan gum	No	No Change	No	No Change	No	No Change

Preparation of gel with HPMC K 100 M:

Accurately weighed quantity of extract was transferred to a beaker and dissolved in 10 ml of propylene glycol into which preservatives were added. HPMC K 100 M was made to disperse in distilled water then heated up to 80-90 with continuous stirring and it was allowed to cool. The 1 %w/v extract loaded propylene glycol solution were added to HPMC K 100 M preparation and stirred vigorously to mix in cold condition and water was added to make up the volume up to 100 ml and stirred in mechanical stirred well to get a uniform gel.

Preparation of gel with Xanthan gum:

Accurately weighed Xanthan gum was taken in a beaker and dispersed in 50 ml of distilled water. Kept the beaker aside to swell the Xanthan gum for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Take 5 ml of propylene glycol and required quantity of Extract. Take 5 ml propylene glycol in another beaker and add weighed quantity of propyl paraben and methyl paraben to it and stirred properly. After all Xanthan gum dispersed, Extract and preservatives solutions were added with constant stirring. Finally volume made upto 100 ml by adding remaining distilled water and Triethanolamine was added drop wise to the formulations for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency.

Composition of Gels

During formulation three gelling agents used at two different concentrations, resulting in six different batches of gels for leaves extract and six batches for root extract, total twelve batches prepared. In this case Carbopol 934, HPMC K 100 M and Xanthan gum, these three types of gelling agents were taken.

Three gelling agents were used as follows:

a. Carbopol 934 (at concentration 1% and 1.5%)
b. HPMC K 100 M (at concentration 1% and 1.5%)
c. Xanthan gum (at concentration 1% and 1.5%)
d.

All the batches were prepared according to the experimental design.

Table no. 2: It shows Quantitative composition of leaves extract gel formulation

Composition(%w/w)	F_1	F_2	F_3	F_4	F_5	F_6
Leaves extract	1	1	1	1	1	1
Carbopol 934	1	1.5	-	-	-	-
HPMC K 100 M	-	-	1	1.5	-	-
Xanthan gum	-	-	-	-	1	1.5
Propylene glycol	10	10	10	10	10	10
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0.5	0.5	0.5	0.5	0.5	0.5
Purified water q.s.	100	100	100	100	100	100
Menthol oil	0.1	0.1	0.1	0.1	0.1	0.1
Triethanol amine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

For leaves extract and root extract there is same experimental design used which results in total 12 gel batches into which 6 batches for leaves extract and 6 batches for root extract.

Table no. 3: It shows Quantitative composition of root extract gel formulation

Composition(%w/w)	F_1	F_2	F_3	F_4	F_5	F_6
Root extract	1	1	1	1	1	1
Carbopol 934	1	1.5	-	-	-	-
HPMC K 100 M	-	-	1	1.5	-	-
Xanthan gum	-	-	-	-	1	1.5
Propylene glycol	10	10	10	10	10	10
Methyl paraben	0.2	0.2	0.2	0.2	0.2	0.2
Propyl paraben	0.5	0.5	0.5	0.5	0.5	0.5
Purified water q.s.	100	100	100	100	100	100
Menthol oil	0.1	0.1	0.1	0.1	0.1	0.1
Triethanol amine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Evaluation of formulated gels

Prepared gels formulations were evaluated for physical appearance, pH, viscosity, spreadability, extrudability.

Physical observation: Transparency and Homogeneity were observed.

Determination of pH: The pH of the gel was determined using a calibrated digital pH meter. The readings were taken for average of 3 samples**.**

Viscosity determination or Rheological studies:

The resistance of a substance to flow is called viscosity. It was measured in Pascal seconds or poises. The rheological studies were determined using a Brookefield Viscometer. All measurements were performed in triplicate.

Determination of Spreadability[11]: 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 on slip and drag characteristics of gels. An excess of gel (about 2 gm) 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 one kg weighted was placed on the top of the two slides for 5 min. 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 gm. With the help of string attached to the hook and the time (in sec.) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability. Spreadability was calculated using the following formula**:**

$$S = M \times L / T$$

Where, S= Spreadability, M= weight in the pan (tied to upper slide), L= Length moved by the slide, T= Time. All measurements were performed in triplicate (n = 3).

Extrudability[12]: The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair). All measurements were performed in triplicate.

In-vitro skin irritation study:[13]

HET-CAM (Hen's Egg Test on the Chorioallantoic Membrane)

Preparation of test system:

Fertile 50-60 g eggs were selected and candled. The eggs which were defective were discarded.-

Incubation of the egg's for 9 days:
Eggs should be rotated at least 5 times daily for the first 8 days of incubation. Incubation for additional 24 hours without rotation. Prior to use removal of the eggshell along the air cell by means of a saw. Moistening of the white inner membrane with 0.9 % (w/v) Nacl solution; keeping egg's warm until use.

Carefully removal of the inner membrane immediately prior to use (not more than 20 min after removal of the egg shell).

- **Treatment with the test substance:**
- Application of test substance directly to the CAM
- Exposure of the CAM to the test substance for at least 300 sec.
- **End point measured by visual inspection:**
- **Haemorrhage:** Bleeding out of the blood vessels of the CAM with red blood dots around the vessels.
- **Lyesis:** Optical disappearance of small blood vessels in the CAM cave. This is not a real Lyesis according to principles of general pathology.
- **Coagulation:** Thrombosis (Intravascular dark spots), extravascular blood coagulation (dark spots), denaturation of albumin.

In-vitro anti-inflammatory activity of prepared herbal gel:
The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate-buffered saline (PBS, pH 6.4) and 2 ml of gel solution so that final concentrations become 31.25, 62.5, 125, 250, 500, 1000µg/mL. A similar volume of double distilled water served as the control. Next, the mixture were incubated at 37±20C in a BOD incubator for 15 minutes and then heated at 700C for five minutes. After cooling, their absorbance was measured at 660nm by using the vehicle as a blank. Diclofenac sodium in the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2500µg/mL) was used as the reference drug and treated similarly for the determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition= 100× [Vt/Vc-1]

Where, Vt = absorbance of the test sample
Vc = absorbance of control

Stability study:
The optimized gel formulations were prepared; packed in aluminum collapsible tubes and subjected to stability studies at 400 C/75% RH for a period of 3 month as per ICH Guidelines. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance, pH, rheological properties, spreadability and extrudability.

RESULTS AND DISCUSSION

The *Clerodendrum serratum* leaf and root extract gel was translucent in appearance and gave smooth feel on application which was maintained after tested stability study. The pH were maintained throughout the study at 7.0 for all the formulations, compatible with the normal pH range of the skin and would not produce any skin irritation. There was no significant change in pH values as a function of time after 60 days of preparation. The measurement of pH of the formulations is necessary to detect alterations during time storage, ensuring that the pH value is compatible with the components of the formulation and with the application place, avoiding irritation.

Viscosity: The initial viscosities of developed gels were measured using Brookfield viscometer with spindle. **(Table no.4)**

Further stability test for three month has been carried out and it was found that viscosity of gel formulation varies from 3671-4237 cps for leaves extract gel and 3549-4215 cps for root extract gel,

Spreadability: Spreadability of all gel formulation varied from 18.36-22.16 gm.cm/sec for leaves extract gel and 18.45-23.61 gm.cm/sec for root extract gel. Marketed Diclofenac gel shows the spreadability 24.82 gm.cm/sec **(Table no.5)**

Table no. 4: It shows Viscosity of Gel formulations (mean ±S.D, n=3)

Batches	Viscosity(cps)	Batches	Viscosity(cps)
F_1	4237± 0.11	F_7	4215± 0.54
F_2	4124± 0.43	F_8	4183± 0.75
F_3	3714± 0.21	F_9	3815± 0.98
F_4	3688± 0.69	F_{10}	3664± 0.15
F_5	3671± 0.58	F_{11}	3549± 0.65
F_6	3984± 0.25	F_{12}	3684± 0.27

Table no. 5: It shows Spreadability of gel formulations (mean ±S.D)

Batch	Spreadability (gm.cm/sec.)	Batch	Spreadability (gm.cm/sec.)
F_1	18.36± 0.4	F_7	18.45± 0.3
F_2	20.06±0.3	F_8	19.54± 0.1
F_3	19.37±0.2	F_9	20.59± 0.3
F_4	21.38± 0.4	F_{10}	20.29± 0.3
F_5	22.16± 0.3	F_{11}	23.61±0.4
F_6	19.45± 0.5	F_{12}	21.44± 0.2

Spreadability of marketed gel:

Table no.6: It shows Spreadability of marketed gel formulations (mean±S.D)

Marketed gel	Spreadability
Diclofenac Sodium Gel	**24.82± 0.2**

Extrudability: Extrudability of all the formulations is higher than 80%. So it can be said that spreadability and extrudability of all the formulations shows good acceptance properties. **(Table no.7)**

Table no. 7: It shows Extrudability of Leaves extract gel formulations (mean S.D., n=3)

Batches	Wt. of formulation in tube (gm)	Wt. of gel extruded (gm)	Extrudability amount (%)	Appearance
F_1	16.77	15.06	89.8	++
F_2	17.52	14.51	82.81	++
F_3	17.06	14.6	85.58	++
F_4	16.78	14.44	86.05	++
F_5	17.5	16.05	91.71	+++
F_6	17.56	15.8	89.97	++
F_7	16.65	14.83	86.06	++
F_8	17.02	15.18	89.18	++
F_9	17.51	14.3	81.66	++
F_{10}	16.85	14.53	86.23	++
F_{11}	17.43	15.86	90.99	+++
F_{12}	17.39	15.42	88.67	++

Optimization of Batch:

After analysis of all batches of formulations for their evaluation parameters like pH, Viscosity, Spreadability, and Extrudability, the formulation batch F5 from leaves extract gel and F11 from root extract gel showed good results. The batch F5 optimized with the good viscosity, Spreadability and Extrudability and F11 shows good spreadability hence, these two batches were used for further evaluation like in-vitro skin irritation study and in-vitro anti-inflammatory study.

The parameters from batches shows in table no.8

Table no. 8: It shows Evaluation parameters of optimized batches

Optimized Batch	pH	Viscosity (cps)	Spreadability (gm.cm/sec)	Extrudability (%)
F_5	6.8	3671±0.58	22.16± 0.3	91.71
F_{11}	6.8	3549± 0.65	23.61± 0.4	90.99

Skin irritation study:-

Absence of skin irritation in gel formulation is acceptable by patient. Skin irritation test performed by using in-vitro skin irritation test method. There are certain problems associated with the use of animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available. Hence, in the present study in-vitro skin irritation method were used to skin irritation study of optimized gel. All gel formulations were found to be free from irritation. Observation indicates acceptability of these gels for topical use. End point measurement for skin irritation test shows in table no.9

Table no. 9: It shows End points for Skin irritation

End Point	Observation
Hemorrhage	-
Lyesis	-
Coagulation	-

Evaluation of the in-vitro anti-inflammatory topical activity:

This study was conducted by applying in-vitro anti inflammatory study by using % inhibition of albumin. The anti inflammatory action of formulation F5 and F11 was calculated and it was compared with marketed preparation (Diclofenac sodium gel). The % inhibition of marketed formulation and both gel formulations are given in table no.22 and 23. The statistical and graphical analysis of results shows that there was no significant difference in the inhibition of inflammation in between the gel F5, F11 and marketed gel. So the prepared herbal gel formulations are as effective as marketed formulations. Anti-inflammatory activity of batch F_5 and F_{11} shows in table no.10 and 11

Table no. 10: It shows anti-inflammatory activity of batch F_5

Concentration µg/ml	Absorbance	% inhibition
Control	0.1987	-
1000	0.3417	71.96
500	0.3181	60.09
250	0.2753	38.55
125	0.2636	32.66
62.5	0.2401	20.83
31.25	0.2129	7.14
Diclofenac sodium gel (1000µg/ml)	**0.353**	**77.65**

Table no. 11: It shows Anti inflammatory activity of batch F_{11}

Concentration µg/ml	Absorbance	% inhibition
Control	0.1987	-
1000	0.3324	72.4
500	0.3272	69.7
250	0.3016	56.43
125	0.2961	53.57
62.5	0.2718	40.97
31.25	0.2605	35.11
Diclofenac sodium gel (1000µg/ml)	**0.353**	**77.65**

CONCLUSION

It can be concluded from the present investigation that proper selection of polymers and drug is a prerequisite for designing and developing a transdermal drug delivery. The physical compatibility studies suggest that polymers selected i.e. Carbopol 934, HPMC K 100 M and Xanthan gum were found to be compatible with drug *Clerodendrum serratum*. The varying concentration of the three polymers was found to affect the gel parameters like viscosity and spreadability. Gel formulations prepared with Carbopol 934, HPMC K 100 M and Xanthan gum showed good homogeneity, no skin irritation, good stability and anti-inflammatory activity. However, the Xanthan gum based gel proved to the formula of choice, since it showed the highest percentage of extrudability, good spreadability and rheological properties. Formulation F5 with 1 % leaves extract and F11 with 1% root extract of *Clerodendrum serratum* showed the best formulation with significant anti-inflammatory activity. Formulation F5 and F11 shows approximately equal anti-inflammatory activity. Hence, there is no need to used roots for the preparation of medicines for anti-inflammatory action.

ABBREVIATIONS:
HPMC : Hydroxyl methyl propyl cellulose
gm : Gram
nm : Nanometer
% : percentage
rpm : Rotation per minute
ml : milliliter

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