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DEVELOPMENT AND EVALUATION OF A NEW PLANT-DERIVED TOPICAL FORMULATION

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ABSTRACT
This research looks at the work of herbal medicine. It is a form of different treatment for several disorders and plant developed drugs which are adding popularity both in developing and developed countries due to their natural origin and less side results in the last few years. The review of the selected plant *Calanthe triplicata* provides evidence for its medicinal properties. The main objective of this work is to formulate a new herbal gel with the capability of topical drug delivery. Formulations using different excipients by changing the polymer ratio are prepared. All the five formulations are appraised to facilitate spread ability, extrude ability, pH, viscosity, diffusion, drug content determination and *in-vitro* skin irritation test. Among the five formulations, F_3 is selected as a best formulation based on the values of evaluation parameters.

KEYWORDS
Herbal, Formulations and Parameters.

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INTRODUCTION
In general, humans have been using many groups of plants medicinally for centuries. Through individual experience and information accepted for generations, indigenous people have gain knowledge about plants may help in lack of complaints of certain ailments. Many patients look for matching and substitute medicine choices in coping with this unbearable disease. Research has pointed out that people suffering from never-ending pain, and those dissatisfied with existing treatment are very likely to seek alternative cure. The medicinal value of herbs are formulated in particular current dosage forms, such as Tablets, Capsules, Topical cream, Gel, Ointment and even some novel drug delivery forms, like extended release, sustained release, and microen capsules

dosage forms. Exclusive rights of herbal formulations have improved over the earlier period and exact evidence of therapeutic action has been accounted by achieving special experiments (*in-vitro* and *in-vivo*)[1].

MATERIAL AND METHODS[2-8]

Maltol, carbopol, hydroxy propyl methyl cellulose (HPMC), glycerin, methyl paraben, propyl paraben, triethanolamine, ethyl acetate, hydrochloric acid, sodium hydroxide were analytical grade. Methanol and water were HPLC grade.

Mouse L929 cell line, Dulbecco's minimum essential media (DMEM), Foetal bovine serum (FBS), 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), 100 U/ml penicillin, 100µg/ml of streptomycin, trypsin – ethylene diamine tetra acetic acid solutions, phosphate buffer solution (PBS), dimethyl sulphoxide, poly ethylene glycol. The selected plant *Calanthe triplicata* was collected and authenticated.

Method of preparation of *Calanthe triplicata* gel (CTG)

Some proportions of Carbopol 934 and HPMC were diffused in 50 ml of distilled water with uninterrupted stirring. Required quantity of methyl paraben and propyl paraben were dissolved in 5 ml of distilled water by heating on water bath.

After cooling, glycerin was added and combined it with original solution. Required quantity of ethyl acetate extract of *Calanthe triplicata* plant was combined to the mixture and the volume was prepared up to 100 ml with distilled water.

All the constituents were combined correctly with constant stirring and triethanolamine was put in drop wise to the formulation to get the skin pH (6.8-7) at required consistency.

Extrudability

A clean, lacquered metal collapsible tube was taken and the prepared formulation was filled in with a nasal dispense of 5 mm gap, extrudability was concluded by quantifying the gel extruded through the gap as soon as a constant load of 1 kg was placed. It was accumulated and weighed up. The percentage of gel extruded was estimated. The extrudability was considered by using the following formula

Extrudability = mass of extrude gel from tube (in gm) / Area (in cm^2).

Swelling Index

The concentration of the carbopol, ionic power and the existence of water are important for the bulge of the polymer. About1 gm of the formulation was placed on permeable aluminum foil and it was transferred into a beaker holding 10 ml of 0.1 N sodium hydroxide. The samples at different time periods were removed from beakers and locate it for a moment to dry and reevaluated. Swelling index was estimated as follows

Swelling Index (%) = $[(Wt - Wo) / Wo] \times 100$

Where,

Wt = Weight of distended gel later time t,

Wo = Standard weight of gel at initial

In-vitro Diffusion Study

About 1.0 gm of gel was reserved in a compartment (Franz diffusion cell). The whole covering was getting in touch with a section which holding 85 ml of 0.1 N sodium hydroxide. The receptor compartment was continuously stirred (100 rpm) using a magnetic stirrer. The temperature maintained was 37 ± 1° C. The study was carried out for 10 hrs with the interval of 0.5, 1, 2, 4, 6, 8 and 10 hrs. The sample was reserved at predetermined time and same volume was replaced with fresh 0.1 N sodium hydroxide. The absorbance of withdrawn sample was measured at 274 nm.

Drug Content Determination

Shimadzu double beam UV spectrophotometer (Lamda 25) with 1 cm matched quartz cells was used for the studies. Drug content of the gel was determined by dissolving accurately weighed 50 mg of standard drug (Maltol) and sample in 0.1N hydrochloric acid and transferred into a 250 ml volumetric flask, diluted to volume with 0.1N hydrochloric acid. From the solution, 5 ml was transferred into a 100 ml volumetric flask, diluted to volume with 0.1N hydrochloric acid. The absorbance of both the standard and sample were recorded at 274 nm using UV- visible spectrophotometer.

In-vitro skin irritation test

Skin irritation is a reversible inflammatory response produced by the arachidonic acid cascade and cytokines in the viable keratinocyte and fibroblast

of the skin. The skin irritation probable of topical formulation is examined prior to the individual exposure to find out the chemicals which might stimulate undesirable skin reactions. Fibroblasts derived from mouse L929 cell line (ATCC L929) were used due to their accuracy of cytotoxicity evaluation.

Cell Culture

Mouse L929 cell line was developed in DMEM high glucose medium containing 10 % FBS and antibiotics (penicillin 100 U/ml and streptomycin 100µg/ml) and regularly cultured into 25 cm^2 plastic flasks and in a humidified incubator at 37˚C with 5% CO and 95 % humidity. The cells were produced with trypsin- EDTA and seeded 100µl/well of a suspension (density of 2×104 cells/ml) into a 96-well plate and incubated for the night.

Drug Treatment

The sample was dissolved in 2 % Poly ethylene glycol solution in DMEM. The appropriate cell culture medium was diluted with 2 % FBS to get the concentrations ranging from 1000µg/ml to 6.25µg/ml. The cell culture medium from 96-well plates was aspirated and each dilution (100µl) was added to the plates in triplicates. The cultures were incubated for 24 hrs and then cytotoxicity assay was carried out.

Cytotoxicity Evaluation

Cell viability was judged by MTT assay. After the drug treatment (24 hrs), about 10µl of MTT solution was added and incubated for 3 hours at 37˚C. The apparatus were washed once with PBS and 100µl/well of dimethyl sulphoxide was added to suspend the purple for mazan product and the absorbance of the resulting solution was measured at a wavelength of 540 nm in a micro plate reader.

RESULTS AND DISCUSSION

The herbal gel was prepared and subjected to evaluation of the various parameters. During the trial, the concentration of carbapol was gradually decreased and HPMC was gradually increased. All the batches (F1 – F5) showed good appearance as well as homogeneity; from the results it was restricted to one batch (F3) batch which showed good outcome for all the gel parameters. Other batches (F1, F2, F4 and F5) were lack in these parameters. The physical parameters like color and appearance were observed and shown in Table No.1. The color of the prepared herbal gel was greenish and the appearance of gel was translucent and also it was smooth on application. The pH values of gel formulation were studied at room temperature and there was no change in pH was observed.

In general, the proper application of gel is depended on their functional properties such as viscosity. The result of viscosity of F3 formulation showed good results and were shown in Table No.2.

The values of spread ability indicate that the gel is simply spreadable by small amount of shear. The result spread ability of F3 formulation showed good results. In delivery of preferred amount of drug from the container, the detection of extrudability becomes a vital decisive factor. The F3 formulation had an excellent extrudability. The results were shown in Table No.3.

The drug content of maltol in *Calanthe triplicata* gel was found to be 30.98 %. The results were shown in Table No.4.

From the data of swelling index, we have found that F3 formulation had larger percent swelling index. The results were shown in Table No.5.

From the data of drug diffusion study, we have found that F3 formulation releases 80.13 % of drug over a period of 10 hrs. The results were shown in Table No.6.

The Stability checking of F3 formulation was shown in Table No.7. The prepared herbal gel formulation (F3) was subjected to stability investigation. The physical factors were assessed during the study period; the result of study point out that the formulation is physically stable at 40° C + 2° C/ 75% + 5 % RH of CTHG. The results were shown in Table No.7.

***In-vitro* skin irritation test**

The time and concentration dependent drop off in the MTT viability assay by the herbal gel on L929 cells were experiential in this study. It is expressed as percentage reduction of tetrazoilum salt by the mitochondrial enzyme. The results were shown in Table No.8.

Table No.1: Compositions of *Calanthe triplicata* herbal gel (CTHG)

S.No	Ingredient	F_1	F_2	F_3	F_4	F_5
1	Carbopol	3g	2.5g	2g	1.5g	1g
2	HPMC	-	0.5g	1g	1.5g	2g
3	Plant extract	2.5g	2.5g	2.5g	2.5g	2.5g
4	Glycerin	2g	2g	2g	2g	2g
5	0.5% Methyl Paraben	0.02 ml				
6	0.2 % Propyl Paraben	0.002 ml				
7	Triethanolamine	Quantity sufficient				
8	Purified water	Quantity sufficient to 100 ml				

Table No.1: Physical evaluation of CTHG

Batches	Color	Appearance	pH
F_1	Greenish	Homogeneous	7
F_2			
F_3			
F_4			
F_5			

Table No.2: Viscosity of CTHG

S.No	Batches	Torque (%)				Viscosity(centipoises)
		RPM				
		50	75	100	150	
1	F_1	32.6	34.2	36.7	39.5	29317
2	F_2	35.7	37.8	39.6	40.3	21468
3	F_3	49.8	50.3	53.6	57.6	18456
4	F_4	42.8	44.3	46.7	49.3	14567
5	F_5	37.2	38.2	40.6	42.7	11467

Table No.3: Spread ability and Extrudability of CTHG

S.No	Batches	Spread ability (gm.cm/ sec)	Extrudability (%)
1	F_1	13.45	60.76
2	F_2	14.56	68.98
3	F_3	46.87	80.84
4	F_4	35.23	75.65
5	F_5	18.56	58.89

Table No.4: Drug Content of CTHG

S.No	Batches	Absorbance	Drug content (% w/w)
1	F_1	0.108	30.56
2	F_2	0.121	32.76
3	F_3	0.545	50.98
4	F_4	0.334	45.67
5	F_5	0.158	39.78

Table No.5: Swelling Index of CTHG

S.No	Batches	Swelling Index (% Sw) Time (hrs) 1	2	4	6	8	10
1	F_1	12.67	19.56	34.56	47.89	52.45	54.56
2	F_2	13.67	18.42	21.45	32.67	45.78	49.78
3	F_3	18.78	25.68	39.76	53.67	66.87	75.67
4	F_4	15.89	18.95	25.36	32.34	37.79	55.67
5	F_5	12.17	21.56	28.65	31.67	34.98	42.31

Table No.6: *In-vitro* Drug Diffusion of CTHG

S.No	Batches	Drug release (%) Time (hrs) 1	2	4	6	8	10
1	F_1	14.65	16.45	28.89	37.39	42.90	59.89
2	F_2	15.89	14.61	23.47	35.62	49.94	54.76
3	F_3	21.56	29.63	40.87	56.78	69.67	80.13
4	F_4	19.73	21.75	27.34	34.54	45.84	66.78
5	F_5	17.89	20.56	30.91	39.79	49.56	52.67

Table No.7: Stability testing at 40° C $\pm$ 2° C / 75 % $\pm$ 5 % RH of CTHG

S.No	Batches	Months 1 Color	2 Appearance	3 pH
1	F_3	Greenish	homogeneous	7
2	F_4	Greenish	homogeneous	7

Table No.8: Cytotoxic effect of CTHG by MTT assay

S.No	Compound	Conc. (µg / ml)	% Inhibition	CTC_{50} (µg / ml)
1	Standard	1000	84.67	400
		800	76.23	
		400	55.56	
		200	43.23	
		100	25.98	
		50	12.67	
		25	10.45	
		12.5	7.64	
		6.25	3.16	
2	Sample	1000	94.45	300
		800	86.34	
		400	72.78	
		200	53.45	
		100	35.67	
		50	29.92	
		25	23.67	
		12.5	15.86	
		6.25	12.78	

CONCLUSION

The present research work in relation to herbal formulation is evaluated for the safety and efficacy and it was concluded that the external application of the herbal gel probably posses no toxic causes on the skin.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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