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Research Article

Formulation and Evaluation of Polyherbal Gel

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ABSTRACT

Centella asiatica is beneficial in the treatment of wounds and ulcerous skin abnormalities, *Curcuma longa* exhibits remarkable anti-inflammatory and antioxidant activity and *Terminalia arjuna* has antioxidant and astringent property. Rationalizing to this traditional claim, the present study was designed to develop and evaluate polyherbal gel containing alcoholic extract of *Centella asiatica*, *Curcuma longa* and *Terminalia arjuna*. The individual herbs were evaluated for their standard specification according to the Herbal Pharmacopoeia of India. Extracts were prepared by established procedure. Different batches of gel (G1 to G5) were prepared by using varying concentration of Carbopol 934 and 1% alcoholic extracts of *Centella asiatica*, *Curcuma longa* and *Terminalia arjuna*. The prepared formulations were evaluated for physicochemical parameters like PH, viscosity, spreadibility, drug content of formulations and In vitro release study. From results of evaluation batch G3 containing 1.2% of Carbopol 934 was selected for antioxidant study. For antioxidant study further two formulations containing 1% (R1) and 2%(R2) of alcoholic extracts of *Centella asiatica*, *Curcuma longa* and *Terminalia arjuna* and 1.2% of Carbopol 934 were prepared. In vitro antioxidant study carried out by using DPPH (1,1-diphenyl-2- picryl hydrazyl) radicals scavenging activity and ferric reducing power ability (FRPA) assay. This study revealed that herbal topical gel had in vitro antioxidant activity.

INTRODUCTION

World Health Organization (WHO) currently encourages, recommends and promotes traditional herbal remedies in national health care programs because such drugs are easily available at low cost. These are comparatively safe and the people have faith in such remedies. According to study, 70-80% of people living in rural areas are dependent on herbal medicine for the treatment of day-to-day diseases. Ayurveda, Unani and traditional Chinese medicine are the important system of medicines largely based on medicinal plants. Herbal drugs are getting popularized in developing and developed countries. Herbal treatments applied topically have gained considerable attention due to their widespread use and ill-defined benefit/risk ratio. Topical application of gels at pathological sites offers great advantages in a faster release of a

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drug directly to site of action as compared to cream and ointment. Gel formulations are used to deliver the drug topically because of easy application, increase contact time and minimum side effects as compare to other topical preparation and oral administration. The growing popularity of natural and herbal medications, easy avaibility of raw materials, cost effectiveness and too little reported adverse drug reaction, promoted us to develop and evaluate topical polyherbal gel formulation which will possess better activity. *Centella asiatica* (Mandukparni brahmi), is phytochemically rich in triterpenoid saponin glycosides, alkaloids, flavonoid, essential oil. It has been known to be used traditionally for their various therapeutic properties like antibacterial, antimicrobial, antiulcer, in skin disorder, and wound healing activity. *Curcuma longa* is phytochemically rich in essential oil, curcuminoids, curcumin, phytosterol. It has been found to exhibit various activities like antioxidant, anti-inflammatory, antihepatotoxic, anti-ulcer, antibacterial. *Terminalia arjuna* is phytochemically rich in triterpenoid saponin glycosides, tannins, flavonoid, anthocyanins and minerals. It has been reported to be used traditionally for their various medicinal properties like astringent, antioxidant, antifungal and antimicrobial. Despite of the fact that these three herbs are reported to have antimicrobial, antioxidant, anti-inflammatory, antiulcer, astringent and wound healing property, their use and application on the skin surface in the raw form is difficult. Hence, the present investigation was thus undertaken for preparation of polyherbal gel formulation using ethanolic extracts of *Centella asiatica, Curcuma longa* and *Terminalia arjuna*, so as to facilitate their effective use topically. The prepared formulations were evaluated for physicochemical parameters like PH, viscosity, spreadibility, drug content of formulations, In vitro release study and In vitro antioxidant activity.

MATERIALS AND METHODS

Procurement of raw materials

The plants were selected on the basis of their anti-microbial activities and their medicinal uses reported in the literatures. The herbs (Terminalia arjuna, Centella asiatica and Curcuma longa) were purchased from plant drug supplier Sanjivani Aushadhalay, Bhavnagar, Gujarat, India. The marker compound curcumin, from Sigma-Aldrich, Bombay, India while asaiaticoside and arjunolic acid were purchased from Spic Pharma Ltd., Chennai, India. All other chemicals were of analytical grade and used without further purification.

Monographic evaluation of Herbs

The individual herbs were evaluated for microscopy [1,2], (Loss on drying, extractive values, ash values) [4,5,7] and Identification by TLC [3,6] as per the Herbal Pharmacopoeia of India.

Preparation of Herbal Extract

Centella asiatica, Curcuma longa and *Terminalia arjuna* powders each of 100gm were extracted with 500 ml ethanol in Soxhlet apparatus. The solutions were filtered and evaporated to dryness in rotary evaporator at 50^0 C. All of the three extracts were dried in desiccator.

Standardization of extracts by HPTLC [3,6,7,8]

A HPTLC system equipped with linomate V sample applicator and Camag TLC scanner III, using Camag Win CATS software was used to identification of some known active constituents in all the three extracts according to procedures described in literature [9,10,11]. Mobile phases for curcuminoid, asiaticoside and arjunolic acid were chloroform: ethanol: gl. acetic acid (95:5:1), chloroform: Gl. Acetic acid: methanol: water (60:32:12:8), toluene: ethyl acetate: formic acid: methanol (6:3:0.1:1.0) respectively. TLC for curcuminoid was detected at 430 nm without derivatization while for asiaticoside and arjunolic acid TLC were detected at 550 nm and 598 nm respectively after derivatization with anisaldehyde sulphuric acid reagent.

Development of Topical gel Formulations [13,14,15,16]

The topical gel was prepared by cold method. The weigh amount of Carbopol 934 was soaked in water for 24 hrs. and their compositions are given in Table 1. The herbal extracts were incorporated into prepared gel.

Table 1: composition of gel

Ingredients	G1	G2	G3	G4	G5
Drug					
Mandukparni brahmi alc. Extract	1 %	1 %	1 %	1 %	1 %
Turmeric alc. Extract	1 %	1 %	1 %	1 %	1 %
Arjuna alc. Extract	1 %	1 %	1 %	1 %	1 %
Carbopol 934	0.8%	1.0%	1.2%	1.4%	1 6%
Propylene glycol	2ml	2ml	2ml	2ml	2ml
Ethanol	5ml	5ml	5ml	5ml	5ml
Water	42.5	42.5	42.5	42.5	42.5
Triethanolamine	q. s.	q. s.	q. s.	q. s.	q. s.
Total weight	50 gm	50 gm	50 gm	50 gm	50 gm

Evaluation of topical gel formulation: [14,15,16]

a) Drug content:

1 gm gel was dissolved in 25 ml phosphate buffer (pH 7.4) containing 5% ethanol l00ml beaker. This solution was further extracted with 50 ml of chloroform and evaporated this solution up to 25 ml (solution -A). 10 µl of solution -A was applied on an aluminum backed silica gel 60F254 plate. The plate was developed in Toluene: ethyl acetate: formic acid: methanol (6:3:0.1: l .0) (for arjunolic acid) and chloroform: Gl. Acetic acid: methanol: water (60:32:12:8) (for asiaticoside) at 25° C and dipped in anisaldehyde sulphuric acid reagent. Heat it in hot air oven for 15 min at l10°C and then chromatogram was recorded by scanning at 598 nm and 550 nm respectively for arjunolic acid and asiaticoside on a CAMAG TLC scanner. 5 µl of solution- A were applied on an aluminium backed silica gel 60F254 plate. The plate was developed in chloroform: ethanol: gl. acetic acid (95:5:1) at 25° C and chromatogram was recorded by scanning at 430 nm on a CAMAG TLC scanner.

From this the drug content was determined using calibration curve of Asiaticoside, curcuminoid and arjunolic acid.

b) pH:

1 gm of gel was accurately weighed and dispersed in 10 ml of distilled water. The pH of these dispersions was measured using pH meter (Systronics digital- DI- 707).

c) Viscosity and rheological studies:

Viscosities of gels were determined using Brookfield Viscometer. Gels were tested for their rheological characteristics at 25°C using Brookfield Viscometer (DV-III programmable rheometer). The Measurement was made over the whole range of speed setting from 10 rpm to 100 rpm with 30 seconds between two successive speeds and then in descending order.

d) Spreadibility:

For the determination of spreadibility excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 gm weight for 5 minutes. Weight (50 gm) was added to the pan. The time required to separate the two slides i.e., the time in which the upper glass slide moves over the lower plate was taken as measure of spreadibility (S).

$S = m \times l/t$

Where,

m = weight tide to upper slide

l = length moved on the glass slide

t = time taken

e) In vitro release study:

The release rates of active constituents from gels were determined using Franz diffusion cell. The diffusion test was performed using 22 ml phosphate buffer (pH 7.4) containing ethanol, at 37 + 0.5°C. A sample (3 ml) of the solution was withdrawn from the diffusion apparatus at an

interval of every one hr. up to 8 hrs. The samples were replaced with fresh diffusion medium of same quantity. Each sample was further extracted with l0ml chloroform and evaporated this solution up to 5ml (solution-B). 40µl of solution-B was applied on an aluminum backed silica gel 60F254 plate. The plate was developed in Toluene: ethyl acetate: formic acid: methanol (6:3:0.1:1.0) (for arjunolic acid) and chloroform: Gl. Acetic acid: methanol: water (60:32:12:8) (for asiaticoside) at 25° C and dipped in anisaldehyde sulphuric acid reagent. Heat it in hot air oven for 15 min at 1l0°C and then chromatogram was recorded by scanning at 598 nm and 550 nm respectively for arjunolic acid and asiaticoside on a CAMAG TLC scanner. 40 µl of solution -B were applied on an aluminum backed silica gel 60F254 plate. The plate was developed in chloroform: ethanol: gl. acetic acid (95:5:1) at 25° C and chromatogram was recorded by scanning at 430 nm on a CAMAG TLC scanner. From this the cumulative percentage of drug release was calculated using calibration curve of Asiaticoside, curcuminoid and arjunolic acid.

In vitro Anti-oxidant activity of topical gel formulation: [17,18,19]

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radicals scavenging activity:

It is one of the most extensively used antioxidant assay for plant samples.

50 µl of DPPH solution diluted up to 3 ml with methanol & absorbance was taken after 30 minutes at 516 nm for control reading. 150 µl of different concentrations of gels and standard were mixed with 150 µl of DPPH and diluted up to 3 ml with methanol. The mixture was kept in dark for 30 minutes and absorbance was measured at 516 nm after 30 minutes. The absorbance of control reduce dose dependently.

The % reduction was calculated as follow.

% **Scavenging** = (A_A — **A_B)/ A_B × 100**

Where,

AAis the absorbance of the tested sample after 30 minutes.

ABis the absorbance of Control sample.

IC50 is the concentration required to reduce % reduction by 50 %.

Ferric Reducing Power Ability (FRPA) assay:

Ferric reducing ability assay is a technique to determine the total antioxidant power interpreted as the reducing capability.

Different concentration of gels in distilled water was mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml) and the mixture was incubated at 50° C for 20 min. 2.5 ml of 10 % TCA was added to the reaction mixture which was centrifuged at 1000 RPM for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml) and the absorbance was measured at 700 nm.

RESULTS AND DISCUSSION:

Monographic Analysis of Herbs

Table 2 shows the results of monographic analysis of the herbs, performed according to the Indian Herbal Pharmacopoeia and WHO guideline for quality control of herbal raw materials. It was found that the moisture content of all the extract were less than 10%. The extractive values and ash vales of all the extracts were within the pharmacopoeias limit. It indicates the good quality of raw materials.

Table 2. Monographic Analysis of Herbs

	C. longa (%w/w)		C. asiatica (%w/w)		T. arjuna (%w/w)	
Parameters	Obtained Value	Pharmaco poeial Limit	Obtained Value	Pharmaco poeial Limit	Obtained Value	Pharmaco poeial Limit
Loss on drying	7.98±0.52	NMT 12	7.66±0.38	NMT 10	6.75±0.25	NMT 12
Alcohol Soluble Extractive	10.15±2.22	NLT 8	10.37±0.33	NLT 9.5	16.21±0.21	NLT 16

Water Soluble Extractive	13.29±0.25	NLT 9	15.38±0.39	NLT 6	20.25±0.43	NLT 17
Total Ash	7.07± 0.062	NMT 9	22.32±0.32	NMT 19	9.10±0.10	NMT 27
Acid Insoluble Ash	0.51±0.028	NMT 1	6.19±0.23	NLT 6	1.40±0.08	NMT 2

Preparation of Herbal Extract

The extract prepared had different color and odor according to raw materials from which they are extracted.

Standardization of extracts by HPTLC

HPTLC analysis of the herbal extracts was performed and the chromatograms of active constituents and extracts are shown in Figure 1. It was found that the R_f values of the active constituents in the chromatogram of extract were match with chromatogram of active constituents. It indicates the presence of active constituent in extracts. The heights of peaks also indicate the presence of active constituents in significant amount in extract.

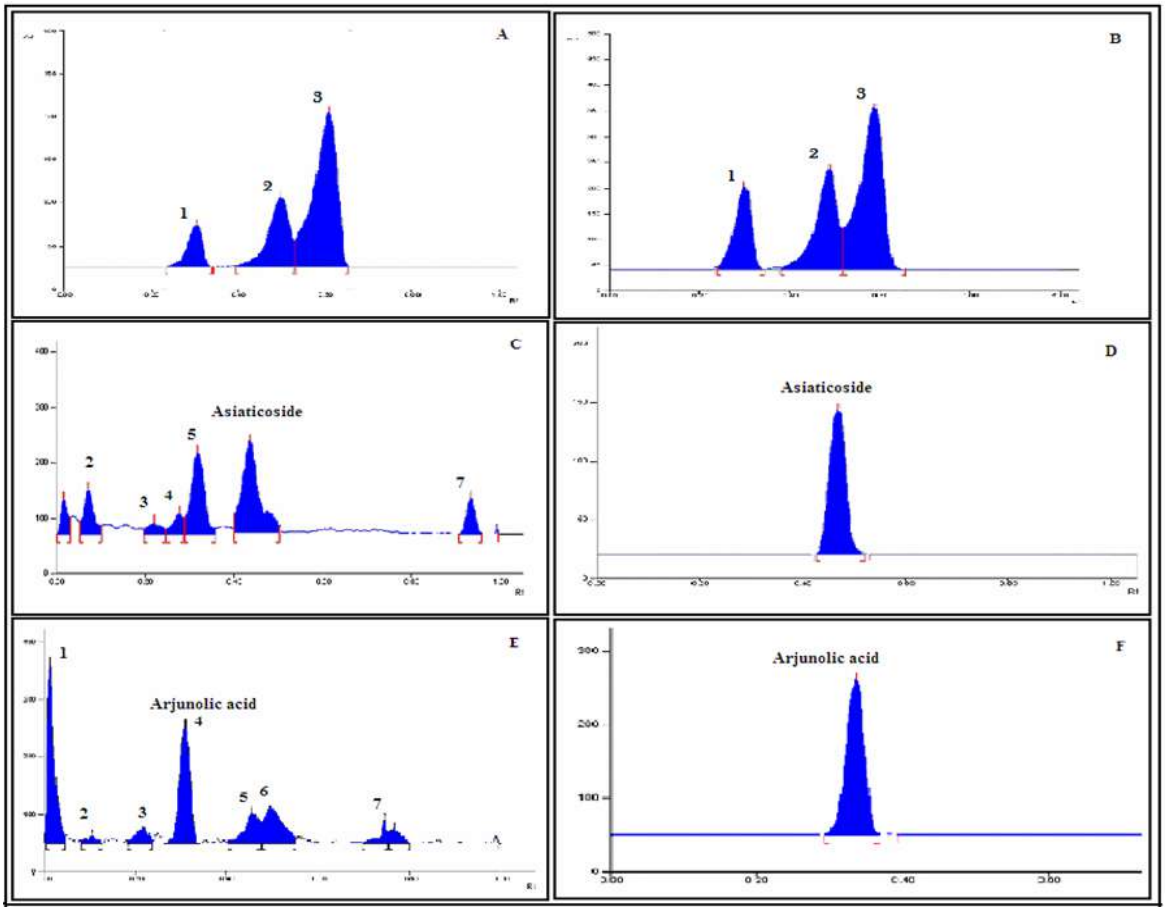


Figure 1. HPTLC Chromatograms of A) *C. longa* Extract, B) Curcuminoids, C) *C. asiatica* Extract, D) Asiaticoside, E) *T. arjuna* Extract, & F) Arjunolic Acid

Development of Topical gel Formulations

Gels prepared with Carbopol 934 were found to be translucent and homogeneous with yellowish brown color as shown in Figure 2.


Figure: 2 Photograph of prepared gel

Evaluation of topical gel formulation:

Asiaticoside, curcuminoid and arjunolic acid content of the formulations were well within the range between 68.31- 80.15% w/w, 79.31-83.46% w/w and 71.4-74.05% w/w respectively (Table: 2) and pH between 5.81-6.6 (Table: 2). Spreadibility and viscosity of various formulation of extract containing gels are shown in Table: 2.

Table:2 Evaluation parameters of extracts containing gel

Batch code	Drug content (% w/w)			pH	Viscosity (cps)	Spreadability Gm*cm/sec.
	Asiaticoside	Curcuminoid	Arjunolic acid			
GI	69.44	79.62	74.05	6.42	587.33×10^3	3.91
G2	76.7	79.31	73.29	6.36	747.46×10^3	1.19
G3	80.15	83.46	74.1	5.9	1240×10^3	0.93
G4	75.67	81.24	74.99	5.83	1363.3×10^3	0.81
G5	68.31	81.05	71.4	5.81	1456.6×10^3	0.74

In vitro drug release profile from topical gel formulations:

The data obtained from in vitro release (Ql and Q6) are shown in Table 3.

Table: 3 In vitro releases (Qland Q6) of asiaticoside, curcuminoid and arjunolic acid from topical gels.

Batch code	Asiaticoside %w/w		Curcuminoid %w/w		Arjunolic acid %w/w	
	Q1	Q6	Q1	Q6	Q1	Q6
GI	*6.5*	58.5	17.5	73.21	7.6	57.6
G2	10.39	63.6	21.87	79.89	9.23	67.13
G3	l 6.3	69.79	31.2	89.7	14.98	71.79
G4	11.58	65.9	29.15	85.36	13.69	65.67
G5	11.35	65.97	25.89	86.71	13.12	65.78

Q1= In vitro release of drugs in lhr Q6= In vitro release of drugs in 6 hr

In *vitro* Anti-oxidant activity of selected topical gel formulation:

1, 1-Dipheny1-2-picryl hydrazyl (DPPH) radicals scavenging activity:

The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or

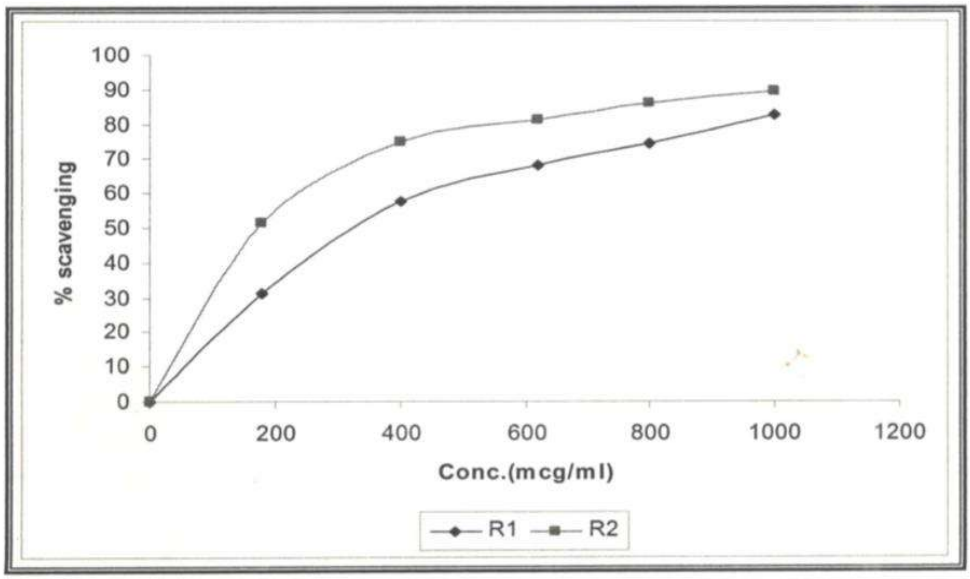
electrons and to capture the free radicals. DPPH analysis was one of the tests used to prove the ability of the components of the topical gel formulation to act as donors of hydrogen atoms. The obtained results were shown in Figure:3. The 2 % extract containing gel (R2) showed a significant effect in inhibiting DPPH, reaching up to 89.5 % at concentration 1000 mcg/ml and its IC50Was 323.59 mcg/ml whereas the 1% extract containing gel (RI) showed 82.9 % of inhibition and its IC50 was 467.93 mcg/ml at same concentration. The IC50value of ascorbic acid was 23.35mcg/ml.

Table 4: IC50 values of samples

Sample	IC 50(mcg/ml)
Ascorbic acid	23.35
1% extract containing gel (RI)	467.93
2 % extract containing gel (R2)	323.59

The present study revealed that the herbal topical gel formulation R1 and R2 had *In vitro* antioxidant activity. As the concentration of extract increases, the free radical scavenging activity also increases and inhibitory concentration decreases.

Figure:3 % scavenging effect of topical gel formulation



Ferric Reducing Power Ability (FRPA) assay:

The 2 % extract containing gel (R2) showed a significant effect in reducing the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue, reaching up to 76.8 % at concentration 1000 mcg/ml (Figure 4) and its EC50was 400.33 mcg/ml (Table 5) whereas the 1% extract containing gel (RI) showed 65.2% of reduction and its $EC_{50}$was 334.95 mcg/ml at same concentration. The EC50 value of ascorbic acid was 238.42 mcg/ml.

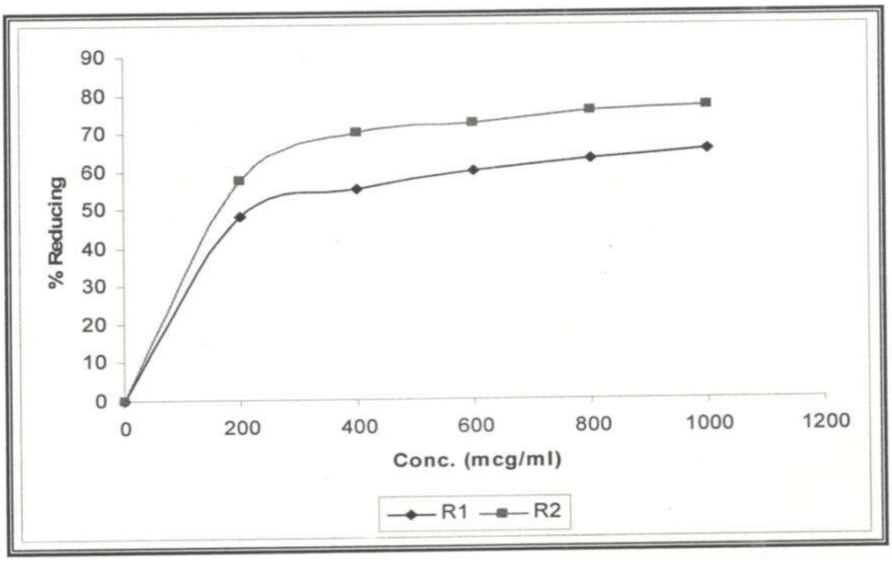
Table:5 EC50 values of samples

Sample	EC_{50} (mcg/ml)
Ascorbic acid	238.42
1% extract containing gel (R1)	334.95
2 % extract containing gel (R2)	400.33

The present study revealed that the herbal topical gel formulation RI and R2 had *In vitro* antioxidant

activity. As the concentration of extract increases, the % reducing activity also increases and effective concentration increases.

Figure:4 % reducing effect of topical gel formulation



CONCLUSION:

For development of effective topical formulations, it is important to determine the release properties of drugs from the semisolid vehicles. Various gel formulations were prepared using different concentration of carbopol 934. From those formulations batch G3 containing 1.2% of carbopol 934 has 65.97%, 86.71% and 65.78 % w/w of in vitro release of Asiaticoside, Curcuminoid and arjunolic acid respectively which was higher than the other batches with better viscosity and Spreadibility. So, batch G3 was selected for anti-oxidant activity of topical gel. The antioxidant study revealed that the herbal topical gel formulation has *In vitro* antioxidant activity. The prepared topical gel hastens the process of destroying the free radicals by acting as an antioxidant.

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