Research Article

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FORMULATION AND EVALUATION OF TOPICAL GEL OF *RUBIA CORDIFOLIA* (MANJISTHA)

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ABSTRACT

The objective of the present study was to formulate and evaluate Topical gel containing Manjistha as a drug. A total of six gel formulations were prepared by homogenization technique using stearic acid, cholesterol, tween 80 and chloroform as excipients. Preformulation studies like UV spectrophotometry and FTIR were performed for the drug. Carbopol was used as a gelling agent in the preparation of these gels. Evaluation studies like pH, spreadability, viscosity, homogeneity and *in vitro* drug release studies were carried out. All the results were obtained to state that prepared gels have shown optimum results. Also, a gel containing stearic acid as a lipid (1:2) has shown maximum drug release compared to other gels.

Keywords: Manjistha, Cholesterol, Stearic acid, Topical gel.

INTRODUCTION

Rubia cordifolia (Rubiaceae) is also known as, Manjishtha, Indian madder, distributed throughout India[1]. It is found throughout the hilly districts of India from the northwest Himalayas eastwards, ascending to 8000 feet and southwards to Ceylon. The roots of this plant are of high medicinal value and are recognized as official[2]. This perennial herbaceous prickly creeper or climber is up to 10m long, found throughout the country ascending to 3750 m and grows well in light (sandy), medium (loamy) and heavy (clay) soils[3].

Rubiaceae comprises about 450 genera and 6500 species and includes trees, shrubs and infrequently herbs. *Rubia cordifolia* L. (Rubiaceae), also known as 'manjistha', it is a perennial, herbaceous climbing plant, with long roots, cylindrical, flexuous, and thin red bark. Stems often have a long, rough, grooved, woody base. Plants belonging to this the family contains substantial amounts of anthraquinones, especially in the roots [4].

The traditional therapeutic use of the plant has been for skin disorders and anticancer activity. Furthermore, the anthraquinones of the Rubiaceae family exhibit some interesting in vivo biological activities, such as anti-tumour, anti-inflammatory, urinary disorders, antistress antimicrobial, hepatoprotective, radioprotective, and anticancer, antimicrobial, antifungal, hypotensive, analgesic, antimalarial, antioxidant, antileukemic and mutagenic functions, immunomodulatory, anti-inflammatory and antioxidant[5].

Apart from its medicinal value, this plant has also been used as natural food colourants and natural hair dyes. The interest in the isolation of natural dyes and colouring, matters are increasing due to their applications in food, drugs and other human consumptions[6]. This plant has also been listed officially as herbal medicine in the Chinese Pharmacopeia for the treatment of arthritis, dysmenorrhea, hematuria and hemostasis, which are free radical related diseases. It has a variety of uses, such as a blood purifier. It is helpful in treating skin diseases, in blood purification, increasing appetite and in stimulation and contraction of the uterus [7].

Antimicrobial action can be characterized as an aggregate term for all active principles that inhibit the development of microscopic organisms, forestall the arrangement of microbial states, and may destroy microorganisms [7].

The primary classes of antimicrobial specialists are disinfectants, which kill a broad scope of organisms on non-living surfaces to forestall the spread of sickness, germicides (which are applied to living tissue and assist with decreasing contamination during a medical procedure), and anti-infection agents (which annihilate microorganisms inside the body). The expression "anti-microbial" initially portrayed just those details from living microorganisms yet is presently additionally applied to engineered specialists, like sulfonamides or fluoroquinolones [7].

Most, as of late, found antimicrobial specialists are altered regular mixtures, and this adjustment is made through substance mode, for instance: b-lactams (penicillin's), carbapenems, or cephalosporin. Unadulterated everyday items, for example, aminoglycosides, and other manufactured anti-toxins, for instance: sulfonamides, are likewise often utilized. The antimicrobial specialists could be named the specialists that can either be bactericidal, which kills microscopic organisms, or bacteriostatic, which delays the development of microorganisms. Antibacterial specialists are the main in battling irresistible sicknesses. However, with their wide use and misuse, bacterial obstruction toward antibacterial specialists has become a significant issue for the present drug industry. Opposition is most regularly founded on formative cycles, for instance, an anti-infection treatment that prompts inheritable resistance [8].

The topical delivery of drugs is an attractive method for local and systemic treatment and is commonly used in treating inflammatory conditions like musculoskeletal injuries and dermatological diseases. There are many advantages to the topical application compared to conventional dosage forms. Especially some systemic severe and adverse effects are avoided [8].

When the drug is delivered topically, it can penetrate deeper into the skin and give better absorption. Topical preparation can be used to prevent the metabolism of the drug in the liver. It can be used to avoid gastrointestinal disorders, risks and inconvenience of intravenous therapy etc. Furthermore, the bioavailability of the drug is increased, and targeted action can be achieved.

The topical delivery with gels can increase the time of presence of the drug on the skin and improve the delivery and release of the substance.

In the present study, an attempt has been made to prepare gels of Manjista using stearic acid and cholesterol as lipids separately.

MATERIALS AND METHODS

Manjistha power was procured from Chaitanya Agro Herbals, Mysuru, as a gift sample. Stearic acid, Cholesterol, Carbopol 934, Triethanolamine, Propylene glycol, and Tween 80 from various sources were of analytical grade.

Analytical method for Manjistha

Calibration curve in pH 6.8 phosphate buffer

A stock solution was prepared from the standard solution to give a 100 µg/ml concentration in ethanol. Aliquots of 0.5, 1.0, 1.5, 2.0 and 2.5 ml from the stock solution were pipetted into 10 ml volumetric flasks. These dilutions gave 5, 10, 15, 20 and 25 µg/ml concentrations of Manjistha. The absorbance of prepared solutions was measured at 540 nm spectrophotometrically against an ethanol blank. The Results and discussion chapter reported standard plot data of Manjistha in ethanol.

Phyto chemical Analysis

Qualitative analysis of phytochemicals

Preliminary phytochemical screening was performed for herbal extract. The presence of various phytochemicals was tested. Different phytochemicals present in both extracts were identified. Procedures and results obtained are given in Table 2. The results state that the extract contained amino acids, alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, and triterpenoids. [9-11]

Fourier transform infrared (FT-IR) spectroscopic analysis

Fourier transform infrared analysis was conducted to verify the interaction between the drug and polymer. The sample powder was dispersed in KBr powder, and pellets were made by applying 4 kg/cm^2 pressure. FT-IR spectra were obtained by powder diffuse reflectance on an FT-IR spectrophotometer type 8400S Shimadzu. [12]

Gel Preparation

An appropriate quantity of carbopol 934 was soaked in water (around 5 ml) for 2 hours. Carbopol was then neutralized with triethanolamine (TEA) with stirring. Then specified drugs and lipids were dissolved in an appropriate and pre-weighed amount of propylene glycol. The solvent blend was transferred to a carbopol container and agitated for an additional 20 min. The dispersion was then allowed to hydrate and swell for 60 min; finally, the pH was adjusted with 98% TEA until the desired pH value was approximately reached (6.8-7). During pH adjustment, the mixture was stirred gently with a spatula until a homogeneous gel was formed. All the samples were allowed to equilibrate for at least 24 hours at room temperature before performing rheological measurements. The formulation chart of the gel is given in Table 1. The prepared gel formulation is shown in Figure 1. [12-14]

Evaluation studies

pH determination: The pH of the gels was determined using a digital pH meter by placing the glass electrode completely into the gel system. The readings were taken an average of 3 times. [15]

Homogeneity: All developed gels were tested for homogeneity by visual inspection after the gels had been set in the container. They were tested for the appearance and presence of any aggregates.

Spreadability test: Place 0.5 gm gel in a premarked circle (1cm diameter) on a glass plate. Another glass plate was placed over the gel, and a weight of 500 gm was placed over this upper glass plate for 5 min. The experiment was carried out in triplicate, and spreadability was expressed in gm.cm/sec. Spreadability can be calculated by using the formula.

$$S = M.L/T$$

Where S = Spreadability, M = Weight tied to upper slide, L = Length of glass slide, T = Time taken to separate the glass slide from each other.

Rheological studies: The rheological measurements were performed on the Brook field viscometer. All measurements were carried out using parallel plate measuring systems having 50 mm diameter and 1 mm gap at 25 ^{0}C. The rheological properties of the formulated gels were studied at different shear rates (rpm), and the viscosity was measured in cP

***In vitro* drug release studies:** The gel was permeated through the dialysis bag. An optimized formulation was selected for these studies. 0.5 gm of gel was placed in the bag in a beaker containing 150 ml phosphate buffer of pH 7.4 and constantly stirred with a small magnetic bead. During the experiment, the temperature was maintained at 37± 0.5 ^{o}C to simulate the human skin condition. 5 ml samples were withdrawn at 0.5, 1, 2, 6 and 12 h and replaced with fresh receptor solution. The samples drawn were analyzed spectrophotometrically at 540 nm. The amount of drugs released was calculated, and the percentage of drugs released was plotted against time.

Table 1: Formulation chart of various gel formulations

Formulation	Drug (mg)	Stearic acid (mg)	Cholesterol (mg)	Tween 80 (%)	Tri ethanolamine (TEA)	Propylene glycol	Carbopol 934	Distilled water
F1	50	50	_	1	1%	0.5 ml	0.5 gm	Q.S
F2	50	100	_	1	1%	0.5 ml	0.5 gm	Q.S
F3	50	150	_	1	1%	0.5 ml	0.5 gm	Q.S
F4	50	_	50	1	1%	0.5 ml	0.5 gm	Q.S
F5	50	_	100	1	1%	0.5 ml	0.5 gm	Q.S
F6	50	_	150	1	1%	0.5 ml	0.5 gm	Q.S

Table 2: Phytochemicals present in Ethanolic extract of Manjistha

Constituents	Test	Observation	Results
Alkaloids	Mayer's test	Formation of the creamy precipitate.	Present
Flavonoids	Lead acetate test	Formation of yellow precipitate	Present
Carbohydrates	Molisch's test	Formation of the violet ring at the junction.	Present
Triterpenoids and steroids	Salkowski test	The presence of steroids is confirmed if the lower layer turns red and that of triterpenoids by the Golden yellow layer at the bottom	Present
Deoxy sugars	Killer killani's test	Formation of blue colour in the acetic acid layer	Absent
Glycosides	Legal's test	Formation of pink to blood red colour	Present
Reducing sugars	Benedict's test	Depending on the amount of reducing sugar present in the test solution appears green or yellow, or red	Absent
Amino acids	Ninhydrin's test	Formation of blue colour	Present

Table 3: Evaluation studies of Formulations F1-F6

Formulation	pH	Spreadability	Viscosity
F1	5.6	0.494	15230
F2	6.2	0.370	14870
F3	5.4	0.492	14400
F4	5.8	0.395	15900
F5	6.0	0.370	15188
F6	5.8	0.518	14203

Table 4: *In vitro* drug diffusion studies (F1-F6)

Time	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
0.5	3.55	6.75	3.46	0.57	8.09	3.99
1	10.68	17.31	13.80	7.35	19.83	11.62
2	22.42	35.21	32.59	20.57	35.95	27.07
4	45.72	56.91	52.64	42.83	56.73	48.21
6	71.52	81.46	75.43	69.27	79.07	73.14

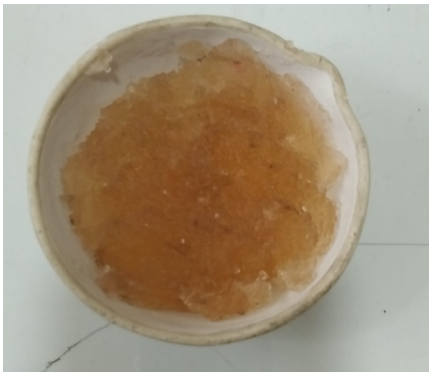

Figure 1: Topical Gel formulation

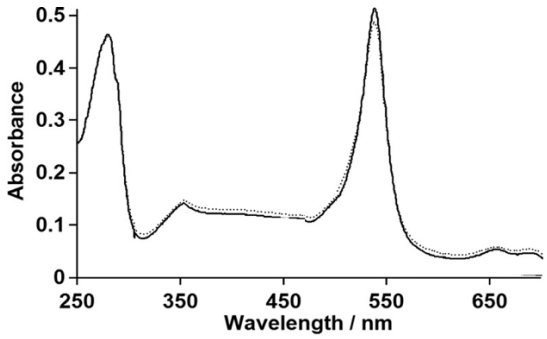

Figure 2: UV-Spectra of Manjistha in 6.8 pH buffer

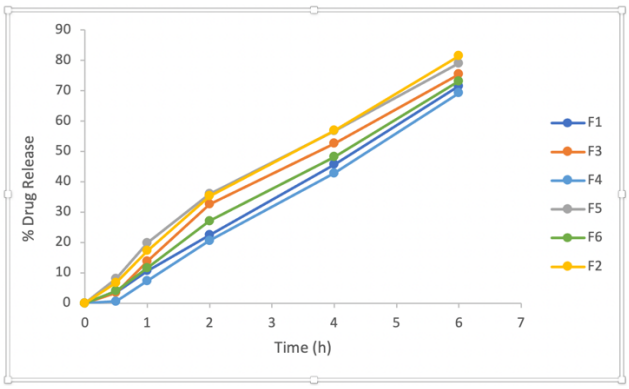

Figure 3: *In vitro* drug diffusion studies (F1-F6)

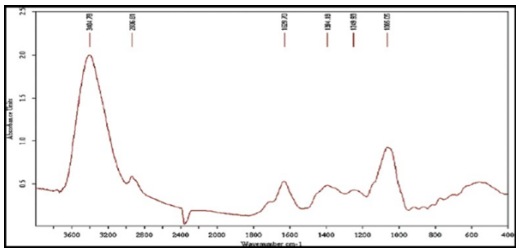

Figure 4: FTIR Spectrum of Pure Drug (Manjistha)

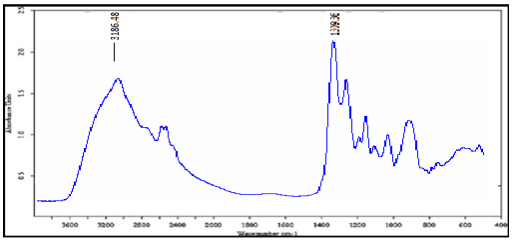

Figure 5: FTIR Spectrum of Formulation (F2)

RESULTS AND DISCUSSION

Calibration curve in 6.8 pH buffer: Standard plot data of Manjishtin in 6.8 pH buffer is reported in Figure 2.

Phytochemical Analysis

Qualitative analysis of phytochemicals: The extract contained amino acids, alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, and triterpenoids. Results are given in Table 2.

Evaluation of gels

pH determination: The pH of the obtained gel was found to be between 5.8 to 6.9, which is near neutral pH. This shows prepared gel does not cause any skin irritation.

Homogeneity: All the prepared gels are clear and transparent. It shows that no aggregates were present.

Spreadability: The spreadability of all prepared formulations was calculated and found to be between 0.3 to 0.5 gm.cm/sec. From the results obtained, it was confirmed that all the prepared gels were spreading easily on the skin.

Rheological studies: Viscosities for prepared gels were between 14000-16000 cps approx. And the viscosities of all formulations were found feasible for topical drug delivery. All the viscosities obtained were almost near since no change in Carbopol quantity was done. The results are shown in Table 3.

***In vitro* drug diffusion studies**: *In vitro,* drug release studies were performed for all formulations. The results obtained in *vitro* release studies were plotted per cent cumulative drug release Vs time and shown in Figure 3. It was found that in the case of F1-F3 formulations where stearic acid was taken as lipid, the F2 formulation has shown maximum *in vitro* drug release and in the case of F4-F6 where cholesterol was the lipid used, F5 has full release. This could be due to the presence of the optimum amount of lipid in both cases. *In vitro* drug release studies of all the formulations are given in Table 4.

Drug compatibility study: Fourier- transform infrared spectroscopy (FTIR) spectra of pure drug and F2 formulation are presented in Figures 4 and 5, respectively.

FTIR spectroscopy was used to identify the functional groups of the active components present in Manjistha based on the peak values in the IR region. FTIR analysis of Manjistha has confirmed the presence of alcohol, phenols, amines, and carboxylic acids. The major IR stretching frequency at 3404 cm^{-1} was due to primary amines. The frequency at 2936 cm^{-1} was due to hydroxyl and aromatic C-H stretching frequency. The band at 1249 cm^{-1} and 1384 cm^{-1} were due to the >C=C< and CH_2 groups, respectively. This together indicates the presence of a carboxylic acid group. The absorbance at 1065 cm^{-1} was due to OH stretching, which suggests the presence of alcohols and phenols functional groups. This result shows that RC does not contain any fatal toxic substances.

FTIR spectrum of the pure drug in F2 Formulation spectra was intact without any significant deviations. Hence there is no interaction between drugs and excipients.

CONCLUSION

the pH of prepared gels was found to be between 5.8 to 6.9, and this indicates the gel causes no skin irritation. The viscosity and spreadability studies were performed, and the results obtained were within limits. *In vitro* drug diffusion studies were performed for prepared gels. Results stated that formulation F2 containing Drug: Lipid in 1:2 ratio has shown maximum *in vitro* drug diffusion compared to other formulations. The FTIR spectra observed that similar characteristic peaks appeared with minor differences in drug and formulation. This indicated no chemical interaction between the drug and the polymers used.

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