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Advancement and Assessment of methanolic extract of Rubia cordifolia for Inflammatory Disorders

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ABSTRACT

The main objective of this work was to test the anti-inflammatory and anti-psoriatic properties of a methanolic extract of Rubia cordifolia L. root in the creation of a hydrogel using several animal models. For hydrogel formulation, methanolic fraction (% w/w) was employed with various Carbopol 940 and sodium CMC combinations. The anti-inflammatory efficacy of the optimized hydrogel was tested in animals utilizing xylene-induced ear edema, croton oil-induced ear edema, and cotton pellet-induced granuloma models. Physical examination revealed that the produced hydrogels were brownish in color and had a homogenous and smooth look when applied. All other test criteria, including as pH, viscosity, spreadability, and consistency, were determined to be acceptable in the F3 hydrogel formulation. As a result, the optimal F3 formulation composition was found to be 1.5 g of Carbopol 940 and 1% sodium CMC. In xylene-induced ear edema in mice, the percent suppression of edema was found to be equivalent to the standard group of therapy (64.69 %). The results of this investigation show that the produced hydrogel of Rubia cordifolia L. has inhibitory effects on acute inflammation. The capacity of croton oil to promote neutrophil influx in mouse ear tissue was shown in this study. Administration with extract hydrogels, as well as Voltaren Emulgel, substantially decreased MPO and NO levels in mice ears (P<0.01). The presence of flavonoids in Rubia cordifolia L. may explain why the methanol extracts have a considerable anti-inflammatory effect.
Keywords: *Rubia cordifolia L, hydrogel, anti-inflammatory, anti-psoriasis, methanolic extract.*

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INTRODUCTION

Medicine from natural source continues to offer health care to more than 80% of the world's population, particularly in underdeveloped countries. Inflammatory diseases induce aberrant inflammation by causing the immune system to assault the body's cells or tissues, resulting in persistent discomfort, redness, swelling, stiffness, and normal tissue damage. Hydrogels are hydrophilic materials that expand when exposed to water. Synthetic copolymers such as polymethacrylate or polyvinyl pyrrolidine are used to make them. In comparison to natural polymers, synthetic polymers are hydrophobic and chemically stronger. *Rubia cordifolia* Linn belonging to family Rubiaceae could be a well-known Ayurvedic herb prevalently known as Indian Madder (English). The Indian Madder of commerce comprises brief rootstocks with various round and hollow, smooth, and straight roots, around the measure of quills. The current study used several kinds of animal models to assess the anti-inflammatory impact of *Rubia cordifolia* Linn *root* extract in the formation of hydrogel based on prior research and literature [1-5]. The stem of *Rubia cordifolia* Linn was chosen based on traditional knowledge and chemical contents mentioned in the literature for inflammatory illnesses. The following actions were used to complete the research study.

MATERIALS AND METHODS

Carbopol 940, sodium carboxy methyl cellulose, methyl paraben, propyl , Triethanolamine, myeloperoxidase (MPO), nitric oxide (NO), naphthyl ethylene diamine dihydrochloride, sulfanilamide, and hexadecyl trimethyl ammonium bromide ,Xylene and croton oil were purchased. All other general reagents and solvents were used of analytical grade. For extraction, dried powdered root of *Rubia cordifolia* were used (150 g each). Plant materials were first extracted with petroleum ether for defatting, and then dried before being extracted with methanol for *Rubia cordifolia*. The presence or absence of distinct chemical compounds was detected by qualitative chemical assays using petroleum ether and ethanol extracts

derived from *root of Rubia cordifolia* L. [6-9]. According to the modified approach of Chirayath et al., five distinct hydrogel formulations containing extract and one control without extract were created [1].

Table 1: Formulations of hydrogel containing methanol extract of *Rubia cordifolia* root (RC)

Ingredients	F1	F2	F3	F4	F5
Carbopol 940 (gm)	0.25	0.5	7.5	1	2
Sodium CMC	5	4	3	2	1
Extract (%w/w)	1	1	1	1	1
Propylene glycol 400 (5%)	5	5	5	5	5
Methyl Paraben (0.5%) (ml)	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (0.2%) (ml)	0.2	0.2	0.2	0.2	0.2
Triethanolamine (ml)	q. s.	q. s.	q. s.	q. s.	q. s.
Distilled water (ml) q.s.	100	100	100	100	100

The following characteristics were used to characterize all of the generated hydrogel formulations like visually, physical characteristics such as the color and look of the herbal gel were examined. pH, spreadability, viscosity, homogenicity and drug content was estimated by measuring the absorbance at 253 nm using a UV/Vis spectrophotometer (Shimadzu UV 1700). For 30 days, test samples of the hydrogel formulation were stored at various temperatures, including 40°C and room temperature. A centrifuge was used to test the stability of the formed hydrogel against centrifugation in 10 ml graded cylinders at 10,000 rpm for 10 minutes (Remi). The formulation that was resistant to centrifugation was chosen for further testing. The anti-inflammatory effect of an optimized hydrogel formulation F3 composition including methanol extract of *Rubia cordifolia* root (RC) is detailed in this study. For anti-inflammatory investigations, healthy Swiss albino mice of either sex weighing 95 to 100 gm were used. The animal care and experimental techniques followed CPCSEA/IAEC guidelines. The animals were placed into three groups, each having three animals. Group I was control, which was applied topically to each animal in the group. Groups II were administered topically made hydrogels of methanol extract of *Rubia cordifolia* root (RC) to each rat. Group III was referred to as standard and provided Voltaren Emulgel (1 %, Diclofenac Sodium; Novartis India Ltd). Ear biopsies were obtained from the control, test, and standard groups and preserved in a 10% buffered formaldehyde solution before being dried and embedded in paraffin. Light microscopy (100x magnification) was used to analyse sections of 5m thickness for haematoxylin-eosin staining and measurement of edoema intensity and leukocyte infiltration [14]. The mouse tail test (Hofbauer et al., 1988) is very well accepted as screening model for measuring antipsoriatic activity of drugs particularly dithranol. This method is based on the induction of orthokeratosis in the epidermal scales of tail of adult mouse which have a normally parakeratotic differentiation has been used for the biological evaluation of antipsoriatic activity of the formulations prepared [4, 7].

RESULT AND DISCUSSION

The dried powdered part of *Rubia cordifolia* were subjected to a standard procedure for determining various physicochemical parameters, including ash values (total ash, acid insoluble ash, and water soluble ash), ash values (total ash, acid insoluble ash, and water soluble ash), and ash values (total ash, acid insoluble ash, and water soluble ash). The swelling index, moisture content (M.C.), and foreign organic matter (F.O.M.) were all calculated. The manufactured gels was brownish in colour, and the appearance of the hydrogel was homogenous and smooth on application, according to the physical examination. Hydrogel formulations comprising methanol fraction of *Rubia cordifolia* root had a pH of 6.92-7.1 and viscosities ranging from 193500 to 196400 cps (RC). The spreadability of the hydrogel formulations was between 15.70-16.96 g.cm/s in case of RC). For the formulation, the % drug release of hydrogels containing methanol fraction of *Rubia cordifolia* (RC) was initially observed to be 7.22%-11.54% (at 15 min.) and 49.88%-68.51% (at 240 min.) respectively for all formulation (F1 to F5 of RC). The standard calibration curve for *Rubiacordone A* methanolic extract at 269nm was mentioned below in Figure 1.

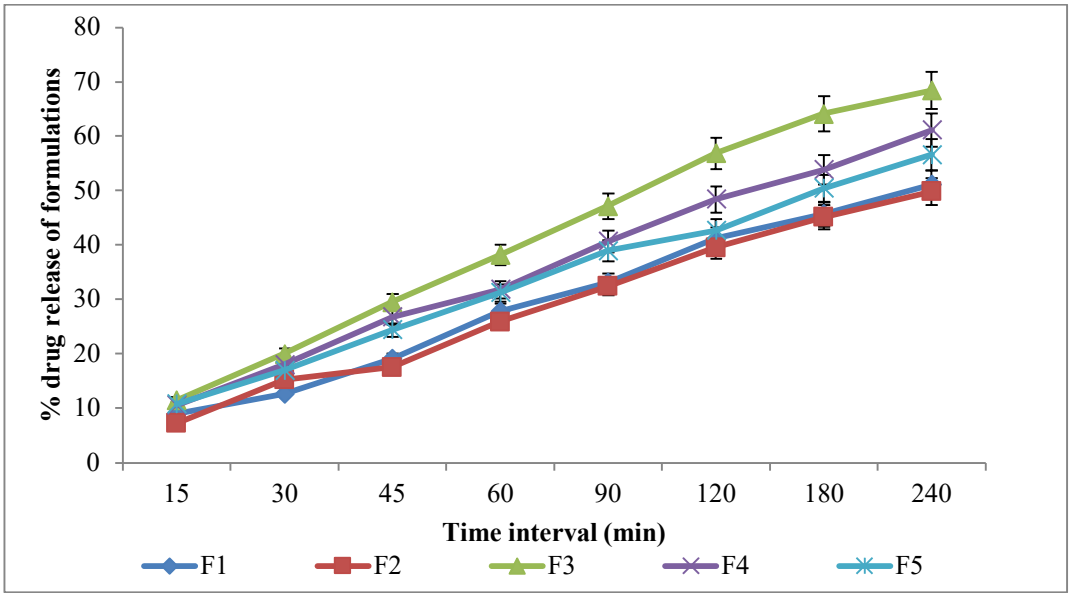

Figure 1: % Drug release of different formulations containing methanol fraction of *Rubia cordifolia*

The optimized formulation, F2, of both plant extracts was applied to rabbit skin, and detrimental effects such as skin colour change and edema were seen by eye. All manufactured hydrogels were subjected to a stability study according to ICH guidelines (2013), These study suggest that the gel was readily spreadable when just a little amount of force was applied. When applied at the target spot, they ensured that the formulation would retain a good wet contact time. The pH of the produced formulations ranged from 6.92-7.1 for RC, which was approximately identical to the pH of skin. As a result, it can be stated that the formulations were sufficient and acceptable in terms of physical characteristics. As a result, the methanolic fractions of *Rubia cordifolia*, were used in hydrogel formulation and anti-inflammatory potential was investigated using chronic proliferative (cotton pellet granuloma) inflammation models (Vogel and Vogel, 1997), xylene induced, and croton induced ear edoema models. As a result, it has the potential to induce ear edoema in mice. RC (1%) hydrogel compositions were used topically in this investigation to see how much xylene produced ear edoema was inhibited in mice (Table 2 and 3).

Table 2: Effect of prepared hydrogel formulations on xylene induced ear edema in mice

Animal groups	Weight of ear lobe (in gm) Mean ± SEM	% inhibition of ear edema
Control (hydrogel base)	0.95±0.05	-
RC (20 mg/day)	0.38±0.10	64.69*
Standard (Voltaren Emulgel)	0.30±0.15	70.24*

Each value is the mean ± S.E.M. (n = 3), *P < 0.05 compared with control and standard. RC: Hydrogel containing methanol fraction of *Rubia cordifolia*

Table 3: Effect of extract hydrogel formulations on Croton oil induced *e*ar edema

Animal groups	Weight of ear lobes (in gm) Mean ± SEM	% inhibition of ear edema
Control (hydrogel base)	1.15±0.15	-
RC (20 mg/day)	0.63±0.25	50.23
Standard (Voltaren Emulgel)	0.69±0.20	55.89

Each value is the mean ± S.E.M. (n = 3), *P < 0.05 compared with control and standard; RC: Hydrogel containing methanol fraction of *Rubia cordifolia.* As shown in the results (Table 4), inflamed mice ears (inflamed control, treated only with placebo gel) had higher NO levels than non-inflamed controls (untreated left ear control) (P0.001), indicating that NO is involved in acute inflammation induced by croton oil in the model used. MPO levels in inflamed ears (inflamed control, treated solely with gel base)

were considerably higher than in non-inflamed controls (untreated left ear control) (P0.01) and the hydrogel formulations treated group. The granuloma development generated by cotton pellets in rats was considerably reduced (P0.05) after treatment with RC (Table 5). This suggests that the anti-inflammatory effect of ethanol extracts is due to inhibition of histamine production, release, or activity. The presence of flavonoids in the methanolic extract of *Rubia cordifolia* may be responsible for the substantial activity.

Table 4: Effect of formulations on inflammatory components (NO and MPO) in Croton oil induced *e*ar edema

Animal groups	Inflammatory components	
	Nitric oxide (NO) level	MPO level
Control (hydrogel base)	21.68±1.10	11.44±0.65
RC (20 mg/day)	14.85±0.55	7.58±0.30
Standard (Voltaren Emulgel)	14.02±0.5	7.98±0.35

Table 5: Effect of extract hydrogel formulations of *Rubia cordifolia* on cotton pellet-induced granuloma in rats

Groups	**Granuloma weight (mg)**	**% Inhibition**
Control (hydrogel base)	62.12± 2.50	-
RC (20 mg/day)	26.31±1.25	59.66
Standard (Voltaren Emulgel)	25.23±1.20	66.82

Each value is the mean ± S.E.M. (n = 3). *P < 0.05 compared with standard. RC: Hydrogel containing methanol fraction of *Rubia cordifolia.* The mouse tail test [3, 6, 12] was applied for measuring antipsoriatic activity of drug dithranol. Healthy male adult Swiss albino mice (25-30 g) were used in this study. Animals were divided into three groups of three each. Different groups designed were as A (control- no treatment), Group B (standard- marketed cream, Derobin, USV Pharma, India), Group C (RC: Hydrogel containing methanol extract of *Rubia cordifolia*). Formulations were applied topically, once daily, 5 times in a week for 2 weeks. After sacrificing animals longitudinal sections of the tail skin were made and prepared for histological examination. In Group A (control- no treatment) orthokeratosis was minimal while It was worth to mention here that both group C had extract containing hydrogel (RC) (Table 6).

Table 6: Antipsoriatic activity of formulated groups (RC)

Treatment Groups	**Relative orthokeratosis (OrthKer)**	**% Relative orthokeratosis (% OrthKer)**	**% Drug Activity**
A. Control	0.9± 0.05	9± 0.5	No drug
B. Standard	0.255±0.017	25.520±1.75	24.427±2.12
C. RC	0.203±0.011	21.980±1.19	20.356±1.50

CONCLUSION

The dried powdered root of *Rubia cordifolia* was subjected to a standard procedure for determining various physicochemical parameters, including ash values (total ash (TA), acid insoluble ash (AIA), and water soluble ash (WSA)). The swelling index (SI), moisture content (MC), and foreign organic matter (FOM) were all calculated. The TA, WSA, AIA, MC, SI, FOM was found 7.025, 4.952, 1.850, 5.681, 0.21, 0.88 for *Rubia cordifolia methanolic extract*. The Rf value represents the spot in the chromatogram or TLC plate where a material was found using the solvent system benzene:chloroform; ethyl acetate and acetic acid for RC extract at varied Rf values ranging from 0.40 to 0.77. The hydrogel was found to be homogeneous and consistent, with no evidence of phase separation. Hydrogel formulations comprising methanol fraction of *Rubia cordifolia* root had a pH of 6.92-7.1 and viscosities ranging from 193500 to 196400 cps (RC). The spreadability of the hydrogel formulations was between 15.70-16.96 g.cm/s in case of RC. For the formulation, the % drug release of hydrogels containing methanol extract of root of RC was initially observed to be 7.22%-11.54% (at 15 min.) and 49.88%-68.51% (at 240 min) respectively for all formulation (F1 to F5 of RC). The optimized formulation, F2, of both plant extracts was applied to rabbit skin, and detrimental effects such as skin colour change and edema were seen by skin irritation tests. The hydrogel formulation F3 of methanol extract of RC was shown to be superior to other formulation combinations and bases in the investigations. The results of this investigation show that the produced

hydrogels RC have anti-inflammatory properties. The mouse tail test [9-13] was applied for measuring antipsoriatic activity of drug dithranol. Healthy male adult Swiss albino mice (25-30 g) were used in this study. In Group A (control- no treatment) orthokeratosis was minimal while It was worth to mention here that both group C had extract containing hydrogel of methanolic extract of *Rubia cordifolia* (RC). The results of Group C were significant when compared with Group B (standard- marketed cream). Group C (RC) had effective drug activity with 20.356±1.50.

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