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Formulation and Evaluation of Novel Herbal Gel of Root Extract of Rubia Cordifolia

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ABSTRACT

The present research has been undertaken with the aim to formulate and evaluate the low cost herbal gel containing *Rubia cordifolia* root extract. The gel formulation was designed by using ethanolic extract of *Rubia cordifolia* root in varied concentrations (750 mg, 1000 mg&1250 mg) and evaluated using physiological measurements. The gel was prepared by using various polymer bases (HPMC K4, HPMC K15, and Carbopol 934). Among them Carbopol 934 has given better gel formation. The gel was prepared by using Carbopol 934, *Rubia cordifolia* root extract, Propylene glycol 400, Methyl paraben, Propyl paraben and required amount of distilled water. Then skin pH (6.8-7) was maintained by drop wise addition of triethanolamine. The physiochemical parameters of formulations (pH, viscosity, spreadability etc.) were determined. Stability studies have carried out as per ICH guidelines for 3 months at different temperatures and humidity. The results showed that formulation containing 750 mg *Rubia cordifolia* root extract have better stability than other formulation. Further all formulations have studied for skin irritation on animal model (Rabbit) and result showed that there was no skin irritation to animals.

Keywords: *Rubia cordifolia*, root extract, Carbopol 934, Gel and ICH guidelines.

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INTRODUCTION

Herbal medicine, forms a major part of traditional medicine, has been used in medical practice since dawn of history and is a common element of ayurvedic, homeopathic, and naturopathic medicine. World health organization (WHO) notes that more 75% of the plant derived medicines are used in modern medicine, in a way that their modern application directly correlates with their traditional use as herbal medicines by native cultures[1, 2]. Rubia cordifolia (Indian Madder, Manjistha) is growing most often near streams and rivers along the upper Ghats in evergreen forests up to 3750 m above sea level. It is a perennial, prickly or scabrous, climbing herb belongs to rubiaceae[3]. Stems is slender, rough, four angled with sharp recurved prickles on the ridges, which are often many yards long, becoming slightly woody at the base. Flowers are in cymes, greenish white. Fruits are didymous or globose, smooth, shining and purplish black when ripe[4]. In ancient world, Manjistha is reputed as an efficient blood purifier and hence is extensively used against blood, skin and urinary diseases[5]. The root is sweet, bitter, acrid, astringent, thermogenic, antidysenteric, anti-inflammatory, antipyretic, analgesic, anodyne, anthelmintic, antiseptic, constipating, diuretic, galactopurifier, febrifuge, rejuvenating and tonic. It is useful in vitiated conditions of *kapha*, the body fluid principles relates to mucus and *pitta*, an energy principle which uses bile to direct digestion. In modern pharmacopoeia, the plant has been used to treat variety of ailments[6,7,8]. The root extract has wide range of pharmacological properties thus used against ailments such as arthralgia, arthritis, cephalalgia, cough, diabetes, discolouration of the skin, dysmenorrhoea, emmenagogue, general debility, haemorrhoids, hepatopathy, intermittent fevers, jaundice, leucorrhoea, neuralgia, pectoral diseases, pharyngitis, ophthalmopathy, otopathy, splenopathy, strangury, slow healing of broken bones, tubercular conditions of the skin and mucous tissue, tuberculosis and urethrorrhoea[9]. Besides, the roots are used for laxative, analgesic, rheumatism, dropsy, paralysis and intestinal ulcers[10]. The roots were used in Ayurvedic (traditional Indian system of medicine) medicine as a colouring agent in medicated oils. Root derived powder has been used in many Asian countries as a natural dye, for imparting shades of red, scarlet, brown and mauve to cotton and other fabrics. Different classes of bioactive compounds such as anthraquinones and their glycosides, naphthoquinones and glycosides, terpenes, bicyclic hexapeptides, iridoids,[11] carboxylic acids (malic, citric, quinic, rosmarinic acids) and saccharides (xylose, ribose, fructose, glucose, sucrose, primverose) were isolated from various parts of *R.cordifolia*. The roots contain a mixture of purpurin, munjistin, small amounts of xanthopurpurin and pseudopurpurin[12, 22]. As part of Ph.D study, successful attempts were made first time in India

to establish the role of Rubia Cardifolia as a potent anti psoriatic agent. The formulated gel is proposed for testing its efficiency in treating psoriasis patients. A gel is a semi solid system of at least two interpenetrating phases, a gelling agent and a liquid. Hydrogels, in the broad view, include the matrix of water-soluble materials such as cellulose derivatives and naturally occurring gums. The Hydrogel is a three-dimensional network of hydrophilic polymer chains that could be cross linked through either chemical or physical bonding. Because of the hydrophilic nature of polymer chains, hydrogels are capable of swelling when placed in aqueous media, *i.e.*, they retain a significant amount of water but remain water-insoluble. When the polymers are cross linked, the hydrophobicity of a gel is increased and the diffusion rate of the drug is diminished. These characteristics of hydrogels, as well as their biocompatibility, increased duration of action with increased therapeutically efficiency due to the viscosity of the gel matrix and soft consistency (easy and safe administration at home by nonmedical persons)[13,14]. Topical application of gels at pathological sites offer great advantage in a faster release of drug directly to site of action, independent of water solubility of the drug as compared to creams and ointments.[15,16]

MATERIALS AND METHOD

Plant Materials

The roots of Rubia Cardifolia were procured from local market and authenticated from Govt Agriculture College, Osmanabad. The root specimen was shade dried and a herbarium sheet is preserved in our college dept for further future references. The roots were shade dried to avoid degradation of phytoconstituents. After drying about 500gm roots were coarsely powdered in a lab mixer, subjected to extraction with ethanol (95%) using Soxhlet apparatus for continuous extraction for 12 hrs, later the extract was cooled at room temperature and evaporation of alcohol afforded a semi solid mass.

Chemicals

Carbopol 934 (Merck Ltd), Methyl Paraben, Propyl Paraben, Propylene glycol-400 and Triethanolamine (SD Fine chemical Ltd).

Preparation of Topical Gel

Different combinations of *Rubia cordifolia* root extract (750 mg,1000 mg &1200 mg) were tried with different types of polymers (HPMC K 4, HPMC K 15 Carbopol 934) using various formulae. The following few combination with Carbopol 934 resulted in the best gel formulation, which was smooth and stable. Control sample also was prepared for testing of animal to check the activity of control ingredients.

Method for Preparation of Gel Containing Root Extract

300 mg of Carbopol 934 was dispersed in 50 ml of distilled water with continuous stirring.5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. Solution was cooled, then to that added Propylene glycol 400. Further required quantity of *Rubia cordifolia* root extract was mixed to the above mixture and volume made up by adding remaining distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. The same method was followed for preparation of control sample without adding any *Rubia cordifolia* root extract.

Formulation

The method describes above and the formulae were tabulated in Table 1. Along with control sample gel were prepared with addition of 750 mg, 1000 mg &1200 mg of *Rubia cordifolia* root extract to prepared *Rubia cordifolia gel* respectively.

Table 1: Different formulations prepared with this ingredients along with quantity.

Ingredient	Control	F I	F II	F III
Rubia cordifolia root extract	-	750 mg	1000 mg	1200 mg
Carbopol 934	300 mg	300 mg	300 mg	300 mg
Methyl Paraben (0.5%)	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Propyl Paraben (0.2%)	0.01 ml	0.01 ml	0.01 ml	0.01 ml
Propylene glycol 400 (5%)	3 ml	3 ml	3 ml	3 ml
Triethanolamine (q.s)	1.2 ml	1.2 ml	1.2 ml	1.2 ml
Distilled water	q.s	q.s	q.s	q.s

Evaluation of Topical Gel Formulation

A. Physical Evaluation

Physical parameters such as colour and appearance were checked.

B. Measurement of pH

pH of the gel was measured by using pH meter.

C. Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end.[17] By this method spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2g) 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. 1 kg weighted was placed on the

top of the two slides for 5 minutes 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 gms. With the help of string attached to the hook and the time (in seconds) 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 the upper slide), L = Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

D. Viscosity

Viscosity of gel was measured by using Brookfield viscometer with spindle.

E. Stability Study

The stability study was performed as per ICH guidelines. [18] The formulated gel were filled in the collapsible tubes and stored at different temperatures and humidity conditions, viz.

$25^0C \pm 2^0C$/ 60% ± 5% RH, $30^0C \pm 2^0C$/ 65% ± 5% RH, 40^0 C ± 2^0C/ 75% ± 5% RH for a period of three months and studied for appearance, pH, viscosity and spreadibility.[19]

Application of Herbal Gel and Skin Irritation Study

Experimental Animals

The experiment was carried out using 5 adult male white rabbits (New Zealand) weighing about 1.5 -2.0 kg to test skin irritation. They were kept carefully following an acclimation period of 7 days to ensure their suitability for present study. Test animals were kept within limited –access rodent facility with room temperature conditions. The experimental protocol was approved by the Institutional ethical committee and care of animals were taken according to the guidelines of CPCSEA. (Registration no-1347/ac/10/CPSCEA).

Preparation of animals prior to testing

The area on the back of each rabbit was shaved prior to the experiment. The shaved areas of the skin of each rabbits were divided into two marked area. The one marked are of respective animals were used for the topical application of developed herbal gel of Rubia Cardifolia and the second area was considered as blank sample for testing the skin irritation as per method of Draize[24]. 0.5 gm of the herbal gel was used as the test substance was applied to an area of approximately 6 cm^2 of skin and covered with a gauze patch. The patch was loosely held in contact with the skin by means of a semi-occlusive dressing for the duration of 1 hour and gauze was removed. At the end of the exposure period, i.e., 1 hour, residual test substance was removed, without altering the existing response or integrity of the epidermis. Observations have recorded after removal of the

patch. Control animals were prepared in the same manner and 0.5 gm of the gel base i.e., gel formulated using all ingredients except the herbal mixture was applied to the control animals and observations were made as similar to the test animals[20]. The gel was applied to the skin once a day for 7 days and observed for any sensitivity and the reaction if any was graded as: [21]

A – No reaction, B – Slight patchy erythrema, C – Slight but confluent or moderate but patchy erythrema, D – Moderate erythrema, E – Severe erythrema with or without edema.

Patch test on healthy human volunteers

As part of Ph.D study Institutional Ethical Committee's approval (Ref No .GACO/SS/5671/2010) and written consent from the patients was obtained prior to the study. The single and repeated patch test was performed on two age groups: in first age group 5 healthy volunteers between 18-24 years while in second group 5 female volunteer age between 18-24 were selected for testing skin reaction /irritation. The dorsal skin of forearm was cleaned with alcohol swab before application of developed gel. The gel was applied with clean finger in circular form with little pressure applied, to ensure penetration of the gel to the 4x4 cm marked region on forearm, and left as it is without covering the region. The formulation was reapplied 3 times for various time periods(repeated test).the cutaneous reactions were evaluated by visual monitoring the reactions of erythrema, edema ,pruritus, skin allergy and irritation at 15 mins,30,min 1 hour and 2 hour, later the forearms were washed with tap water.

RESULTS AND DISCUSSION

The assessment of irritation of pharmaceutical products with natural compounds is a significant step in the evaluation of their biocompatibility. Researcher and regulatory agencies recognize the important role of in vitro and animal tests play in the biologic evaluation of herbal products. The herbal gel was reddish in colour and translucent in appearance and gave smooth feel on application which was maintained after tested stability study (Table 2, 3, 4 & 5). pH also maintained throughout the study which was found 6.92 to 7.0. Spreadability was also measured and found to be less variation with the initially prepared gel after performs the stability study (Table 2, 3, 4 & 5). The initial viscosities of developed gels were measured using Brookfield viscometer with spindle. Further stability test for three months has been carried out and results revealed gel containing 750 mg Rubia *cordifolia* showed better stability than 1000 mg and 1200 mg. Initial viscosity for gel containing 750 mg, 1000 mg and 1200 mg *Rubia cordifolia* extract were 28620 cps, 29726 cps and 30156 cps respectively and after stability study there were not much variation at different temperature and humidity. The gel was non-irritant upon application on to the skin

(Table 6). The control and experimental rabbits showed no signs of tremor, convulsion and reflex abnormalities. The food intake per day had also found normal during 7 days repeated dose dermal toxicity evaluation. No irritation was observed on the healthy human volunteers (Table 7). Ganesh Misal[25] evaluated herbal gel and assessed the anti inflammatory property of herbal gel and the evaluation of gel properties are similar to our findings. Kuntal Das[26] et al studied the formulation and evaluation of herbal gel of stevia extract and the values co relate our values. The developed gel was found to be safe for further use on patients suffering from psoriasis.

Table 2: Physical evaluation of formulations initially (0 month)

Formulation	Control	F I	F II	F III
Colour	White	Reddish	Reddish	Reddish
Appearance	Clear and Transparent	Clear and Translucent	Clear and Translucent	Clear and Translucent
Spreadibility (gm.cm/sec)	16.02	23.15	21.28	19.12
pH	7.10	7.08	7.06	7.06

Table 3: Physical evaluation of formulations at 25^0 C ± 2^0C/ 60% ± 5% RH at 3rd month

Formulation	Control	F I	F II	F III
Colour	White	Reddish	Reddish	Reddish
Appearance	Clear and Transparent	Clear and Translucent	Clear and Translucent	Clear and Translucent
Spreadibility (gm.cm/sec)	15.72	22.85	21.02	18.92
pH	7.08	7.02	7.00	7.02

Table 4: Physical evaluation of formulations at 30^0 C ± 2^0C/ 65% ± 5% RH at 3rd month

Formulation	Control	F I	F II	F III
Colour	White	Reddish	Reddish	Reddish
Appearance	Clear and Transparent	Clear and Translucent	Clear and Translucent	Clear and Translucent
Spreadibility (gm.cm/sec)	15.76	22.32	21.00	18.01
pH	7.05	7.00	6.98	7.01

Table 5: Physical evaluation of formulations 40^0 C ± 2^0C/ 75% ± 5% RH at 3rd months

Formulation	Control	F I	F II	F III
Colour	White	Reddish	Reddish	Reddish
Appearance	Clear and Transparent	Clear and Translucent	Clear and Translucent	Clear and Translucent
Spreadibility (gm.cm/sec)	15.02	22.32	21.00	18.01
pH	6.92	6.93	6.96	6.98

Table 6: Skin irritation study results.(rabbits)

Treatment	Control	F I	F II	F III
Day 1	A	A	A	A
Day 2	A	A	A	A
Day 3	A	A	A	A
Day 4	A	A	A	A
Day 5	A	A	A	A
Day 6	A	A	A	A
Day 7	A	A	A	A

A – No reaction, B – Slight patchy erythrema, C – Slight but confluent or moderate but patchy erythrema, D – Moderate erythrema, E – Severe erythrema with or without edema.

Table 7: Skin irritation study results (Human Volunteers)

Parameters	Single patch test	Repeated patch test			
		15 mins	30 mins	1 Hr	2 Hr
Erythrema	Nil	Nil	Nil	Nil	Nil
Edema	Nil	Nil	Nil	Nil	Nil
Pruritus	Nil	Nil	Nil	Nil	Nil
Skin allergy	Nil	Nil	Nil	Nil	Nil
Irritation	Nil	Nil	Nil	Nil	Nil

CONCLUSION

It is inferred from results that the gel formulations are good in appearance, homogeneity and easily spreadable. As psoriasis being a major cause of morbidity and mortality in patients, these herbal extracts may prevent infection that leads to high risk of scaling, and thereby prevents the prolongation of inflammatory phase of psoriasis. Further study on the fractionation of active components and the mutual effect of these plant extract machinery on psoriatic conditions may provide a better understanding of the infection management in the process of healing.

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