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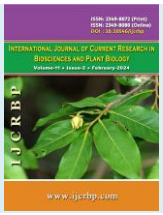

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Characterization formulation and evaluation of emulgel of *Crotalaria juncea* for inflammation pain relief

P. Bhuvaneswari, S. Kasthuri, S. Bhavani, K. Nagalakshmi*

Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology, Chinna Kolambakkam, Maduranthagam-603308, Tamil Nadu, India

*Corresponding author; e-mail: knagalakshmiphd@gmail.com

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Abstract

Emulgel is an emerging drug delivery system. The emulgel was prepared by using carbonol 934 and emulsified agent ratio (span: tween) for geometric and homogenous mixing of all the excipients and the plant extracts. By using slab technique, we have developed five batches of our emulgel, namely F1, F2, F3, F4 and F5 From% drug diffusion study it was observed that, the preparation topical PPE emulgel formulation released a maximum of 83.56 ± 0.544% over a period of 6 hours. The studies of the prepared emulgel were carried out for 60days by keeping at 25°C ±2°C/60%R H 5%±5%, 32°C±2°C and 60%RH±5%R Hand 40°C±2°C and 75%±5% relative humidity (RH). The results indicated different parameters like P^H, viscosity, no phase separation and no significant changes in physical appearance, spredability and drug content observed when compared with the initial formulations, F4 contain maximum result when compared to others. The *in-vitro* release kinetics reveals that all the formulations fit well with zero order kinetics followed by non-Fickian type mechanism.

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Introduction

Crotalaria juncea, known as brown hemp, Indian hemp, Madras hemp, or sun hemp, is a tropical Asian plant of the legume family (Fabaceae). It is generally considered to have originated in India. The preliminary phytochemical screening of the *Crotalaria juncea* leaves revealed the presence of carbohydrates, steroids, triterpenes, phenolic, flavonoids, alkaloids, amino acids, saponins, glycosides, tannins and volatile oils. The plant possesses hypolipidemic, reproductive, antioxidant, antibacterial, antifungal, anti-diarrhoeal, anti-inflammatory, hepatoprotective, and many other pharmacological effects. The plant is native in Asia especially Asia tropical (Bangladesh; Bhutan; India). It is now widely cultivated in the drier areas of the tropics and subtropics and in many temperate areas with a hot summer. It often escaped from cultivation, naturalizes easily and grows in many areas as a ruderal plant. *Crotalaria juncea* is recorded in many countriesThe major significance of Crotalaria juncea lies in its valuable bast fibre, which makes up about 8% of the dry stem weight. The fibre of commerce consists of greyish to pale yellow strands 75–150 cm long. Fibres are

entangled in a mesh structure, and single filaments are obtained by combing and splitting the mesh structure.

Physicochemical properties

The physicochemical properties of *Crotalaria juncea* leaves (% w/w) were: total ash value 5.9, acid insoluble ash 2.7, water soluble ash 3.9, sulphated ash 5.1, moisture content 11, foreign matter 0.04, alcohol soluble extract value 5.84, water soluble extract value 20.4 and crude fibre content. 21C˚): 0.8832, color (lovibond): Y=10.1, R=0.3, acid value: 2.7, saponification value: 217, iodine value: 119.71 and maleic anhydride value: 8.04.

Chemical constituents

The preliminary phytochemical screening of the *Crotalaria juncea* leaves revealed the presence of carbohydrates, steroids, triterpenes, phenolics, flavonoids, alkaloids, aminoacids, saponins, glycosides, tannins and volatile oils Riddelline, seneciphylline, senecionine, trichodesmine, chodesmine alkaloids, galactose-specific lectin and cardiogenin 3-O- [β]-d-xylopyranoside were also isolated from *Crotalaria juncea*.

Materials and methods

Plant identification

Crotalaria juncea plant was collected from local village Malaipalayam village, near Karunguzhi, Madhuranthagam Taluk, Chengalpet district.

Fig. 1: Plant *Crotalaria juncea*.

Preparation of plant extract

Following collecting and identification, the chosen plant was washed with tap water, sliced into smaller pieces, and allowed to dry in the shade for four weeks. The dry material was macerated with methanol after being crushed to powder (100 g) (80:20). An eight-layer muslin cloth was employed for rough extraction after the 72- hour maceration period. The filtrate had been subjected to rotary evaporator with reduced pressure until dry mass obtained. It was maintained between 2 and 8 °C while not in use. Total phenolic content estimation and total flavonoid content estimation were carried out with suitable analytic methods.

Preparation of emulgel

The gel phase in the formulations was prepared by dispersing Carbopol 934 in purified water with constantst irringata moderate speed using mechanical shaker, then the pH was adjusted to 6–6.5 using triethanolamine (TEA). The oil phase of the emulsion was prepared by dissolving span 20inlightliquid paraffin while the aqueous phase was prepared by dissolving tween 20 in purified water. Methyl parabens were dissolved in propylene glycol whereas *crotalaria juncea* extract was dissolved in ethanol, and both solutions were mixed with the aqueous phase. DMSO was mixed in oil phase. Both the oily and aqueous phases were separately heated to 75^0 C, and then the oilyphase was added to the aqueous phase with continuous stirring until it got cooled to room temperature. The obtained emulsion was mixed with the gel in 1:1 ratio with gentle stirring to obtainthe emulgel. The composition of Herbal emulgel formulations is shown in table 2 and 3.

Evaluation of Emulgel:

Physical apprearance

The prepared Emulgel were visually checked for the color, appearance, homogeneity, phase separation and consistency.

pH of emulgel

The pH of the emulgel was measured by a digital pH meter, at a working temperature of 25 ± 1°C. The measurements of pH of each system were replicated three times.

Viscosity

The viscosity of the prepared formulations was determined at ambient temperature using Brookfield digital viscometer (DV-E) with spindle no. 63 at 12, 30 and 50 rpm.

Phase separation

Prepared gel was kept in a closed container at a temperature of 25-100 ℃ away from light. Then phase separation was checked for 24 h for 30 days. Any change in the phase separation was observed and checked.

Greasiness

The gel was applied on the surface in the form of smear and checked if the smear was oily or grease-like.

Determination of spread ability

The spreading coefficient of the formulations was determined using an apparatus consisting of two glass slides (7.5 × 2.5 cm), one of which was fixed onto the wooden board and the other was movable, tied to a thread which passed over a pulley, carrying a weight. Formulation (1 g) was placed between the two glass slides. Weight (100 g) was allowed to rest on the upper slide for 1 to 2 minutes to expel the entrapped air between the slides and to provide a uniform 1m of the formulation.

The spreadability was calculated by following formula,
S=M.L/T
Where, S= spredability, M= weight tied to upper slide, L= length of glass slides, T= time taken to separate the slides completely from each other.

Table 1. Factors, factor levels and responses for Emulgel formulation.

Factors (Independent variables)	Factor levels used		
	Low (-1)	Medium (0)	High (+1)
Amount of carbapol 934(X1)	0.5	1	1.5
Amount of emulsified agent (X2) (Span: Tween 20)	2	4	6
Responses (Dependent variable): Y1= Percent viscosity (% cp); Y2=Percent spreadability (%min/sec)			

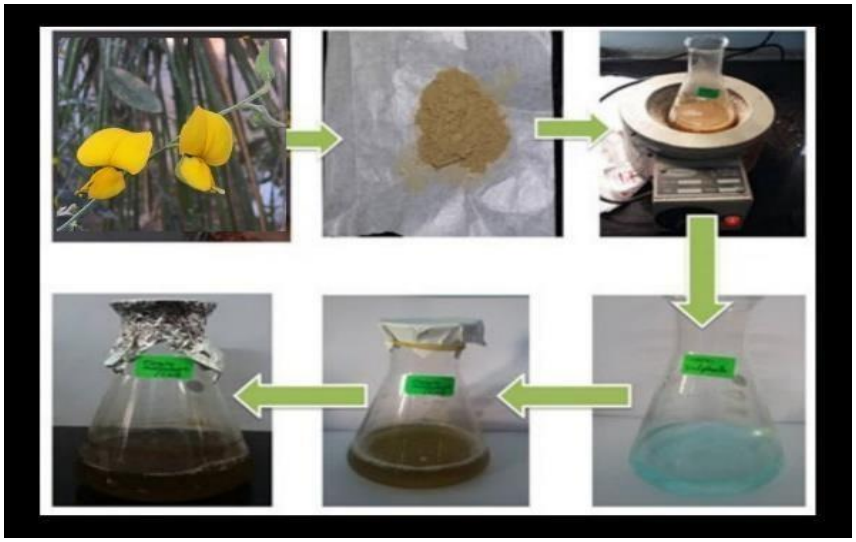

Fig. 2: Plant extraction process.

Table 2. Preparation of Emulgel using following concentration (%W/W).

Ingredient	F1	F2	F3	F4	F5
Carbapol934	0.5	1	1.5	2.0	2.5
Emulsified agent (Span: Tween)	10	8	6	4	2
Liquid paraffin	7.5	7.5	7.5	7.5	7.5
Propylene glycol	5	5	5	5	5
DMSO	0.05	0.05	0.05	0.05	0.05
Methyl Paraben	0.1	0.1	0.1	0.1	0.1
Triethanolamine	q.s	q.s	q.s	q.s	q.s
Water	Up to 100	Up to 100	Up to 100	Up to 100	Up to 100

Table 3. TPC and TFC values of different extracts with different concentration of ethanol solvents.

Ethanol extract	TPC(µgGAE/ml)	TFC(µgQE/g)
50	1.69±0.009	0.165±0.018
70	3.48±0.0027	0.278±0.031
100	5.65±0.0081	0.431±0.017

Results and discussion

Phytochemical analysis of *Crotalaria juncea* plant

The phytochemical analaysis of plant extract shows presence of chemicals such as phenolics, triterpenes, flavonoids, alkaloids, aminoacids, saponins, glycosides, tannins, and voliteoils, positioning it as potential source of antioxidant, anti-inflammatory and antimicrobial agents.

Anti-inflammatory activity in *Crotalaria juncea* plant extract

The Protein denaturation assay method was used to assess the anti-inflammation activity of the *Crotalaria juncea* extract, which was found to be 89.23 ± 0.028 %.

Total phenolic content measurements

Using the Gallic acid standard curve value and the formula y = 0.0026x + 0.4327, R^2 = 0.994, the total phenolic content (TPC) of *Crotalaria juncea* extract was calculated. The TPC value of ethanolic extract from was 5.65µg GAE/ml. The measured TPC values for different concentration 50 ml, 70 ml,100 ml 0fethanolwere1.69µgGAE/ml,3.48µgGAE/mland5.65G AE/ml.

Total flavonoid content measurement

The TFC value of the extracted *Crotalaria juncea* was 8.64±0.0046mgQE/g of AT extract, with the application of the Quercetin standard curve formula (y = 0.0024x + 0.4434, R^2 = 0.9956). The TFC values of ethanolic extract from was 0.43 µgQE/g, The measured TPC values for different concentration 50 ml,70 ml, 100 ml of ethanol were 0.165 µg QE/g, 0.278 µg QE/g and 0.431 µg QE/g (Table 4).

Evaluation of emulgel stability

In general, gel formulation is more preferred, among the topical semisolid preparations, since it has long residence time on the skin, high viscosity, moisturizing Quality control test for formulated topical herbal gel of five gel formulations F1 to F5 (Fig. 4) prepared using carbopol polymers were evaluated for physical appearance, pH, viscosity, spreadability, net content, extrudability and in vitro diffusion profile. From the results of the study for prepared gels were found to be homogeneous and in good appearance and consistency. The pH values of all the formulations were in the close range of neutral pH 5.9 to 6.9 (Fig. 5) and hence it caused no skin irritation, which is also supported by skin irritation study. Polymers were included in the designed topical formulations in order to provide a prompt release of drug and to achieve as well as to maintain the drug concentration within the therapeutically effective range. As the concentration of the polymer was fixed as 2 % in all the gel formulations no big variation in viscosity was observed. Further the value between 2227, 2552, 2713, 2863 and 3014 poise was reported to be an ideal viscosity value for topical gel formulation developed using carbopol polymers (Kim et al., 2003). The greasiness of the gel is normal. Values of the spreadability indicated that the gel formulations are easily spreadable.

In vitro diffusion profile and release kinetics

In vitro diffusion profile of F1 to F5 formulations are recorded. Since the pH of membrane used was in the range of 5.4 to 6.8, phosphate buffer saline pH 7.4 was used for the in vitro release studies of the gel formulations. The in vitro release profiles of all the three formulations made using carbopol 934 elicited almost 100 % release from the formulation within 6 hr. The *in-vitro* release characteristics of the prepared Emulgel formulations were quite encouraging and in agreement with marketed diclofenac gel. Among the formulations, F4 showed better release (83.56%) characteristics than F1to F5. Based on our kinetic release study, we observed that invitro release kinetic study of emulgel formulated with Carbopol 934.

Compatability studies

Compatability study of emulgel was done compare with diclofenac sodium by using IR spectroscopy and IR spectrum was measured in the semisolid state the region in which 4000 to 500.The sensitivity was 75. The

characteristic speaks which are observed in the IR spectrum of the mixture of emulgel are 448.05, 728.04, 1054.38, 1404.78, 1682.24, 2026.79, 3249.05 cm^{-1} while diclofenac sodium gel values are 547.78, 611.43, 680.81, 759.95, 852.54, 933.55,1176.58, 1475.54, 1789.94, 1996. 32, 2044.54, 2620.98, 29868.45, 3064.89, and 3251.77cm^{-1} which are more similar with peak were observed in the IR spectrum of prepared F4 Emulgel. Hence the compatibility study between prepared F4 gel and diclofenac sodium gel are as same so prepared F4 gel will give good results.

Table 4. Evaluation parameters for Emulgel formulation made with 2.0% Carbopol 934.

Code	Conc (%)	pH*	Viscosity* (poise)	Spreadability* gcm/sec	Drug content* %w/w	Extrudability*	Physical appearance
F1	0.5	5.457± 0.51	2227	16.19	60.7±0.53	Fair	Pale yellow, smooth, homogenous and translucent
F2	1.0	5.857± 0.73	2552	18.05	64.2±0.74	Good	Pale yellow, smooth, homogenous and translucent
F3	1.5	6.079± 0.18	2713	24.89	76.13±0.53	Excellent	Pale yellow, smooth, homogenous and translucent
F4	2.0	6.617± 0.28	2863	26.41	83.56±0.54	Excellent	Pale yellow, smooth, homogenous and translucent
F5	2.5	6.8±0.18	3014	27.13	78.12±0.32	Good	Pale yellow, smooth, homogenous and translucent

Table 5. Kinetic release studies of formulation F1to F5.

Formulation code	Zero order R2	First order R2	Higuchi diffusion model R2	Best fitted model
F1	0.909	0.891	>1	Zero order
F2	0.914	0.917	0.936	Zero order
F3	0.924	0.933	>1	Zero order
F4	0.953	0.941	>1	Zero order
F5	0.905	0.971	0.917	Higuchi

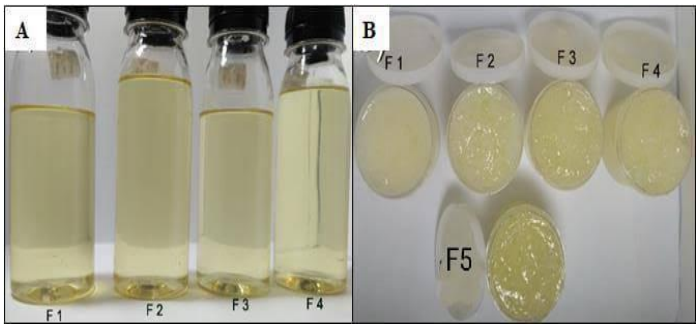

Fig. 3: Preparation and evaluation of Emulgel.

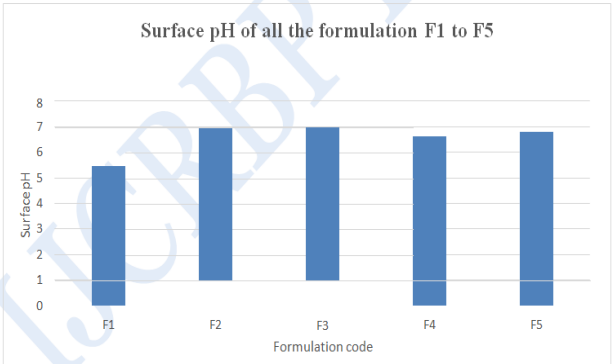

Fig. 4: Surface pH of all the formulations F1 to F5.

Fig. 5: Stability studies of prepared F4 Emulgel.

Table 6. Stability studies of Emulgel formulation F4 results.

Parameters	**Storage condition (temperature)**		
	25^0C±2^0C/60 %RH±5%R H	**32^0C±2^0C/60 %RH±5%R H**	**40^0C±2^0C/60% RH±5%RH**
Color	No change in colour	No change in colour	No change in colour
Odour	No change in appearance	No change in appearance	No change in appearance
Homogenity	Smooth	Smooth	Smooth
pH	6.579±0.18	6.579±0.18	6.579±0.18
Viscosity (poise)	2863	2863	2863
Microbial load (Bacteria and Fungi)	No growth was observed	No growth was observed	No growth was observed
Sterility test	No growth was observed	No growth was observed	No growth was observed

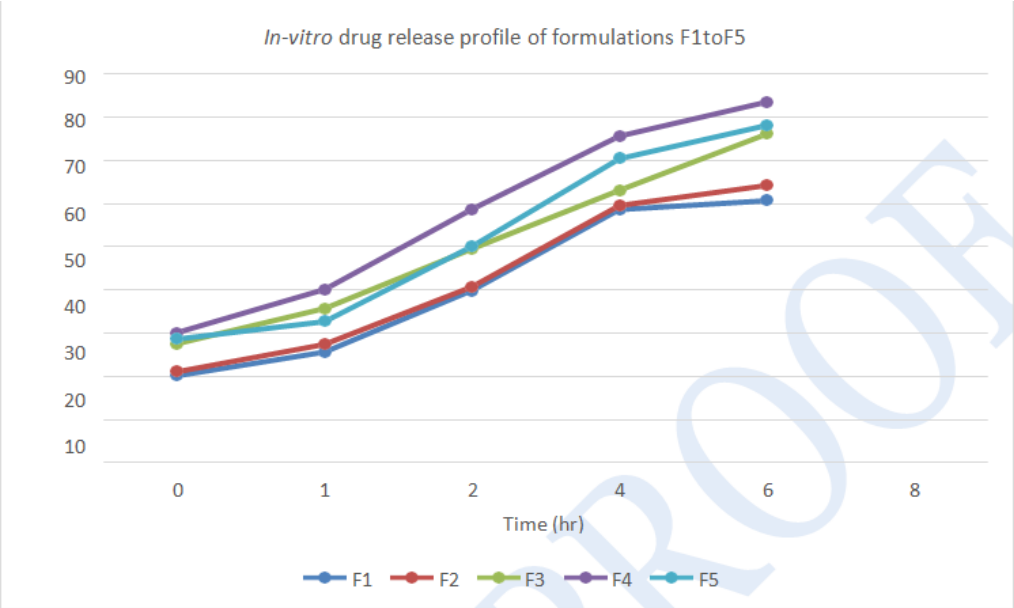

Fig. 6: *In-vitro* drug release profile of formulation F1 to F5.

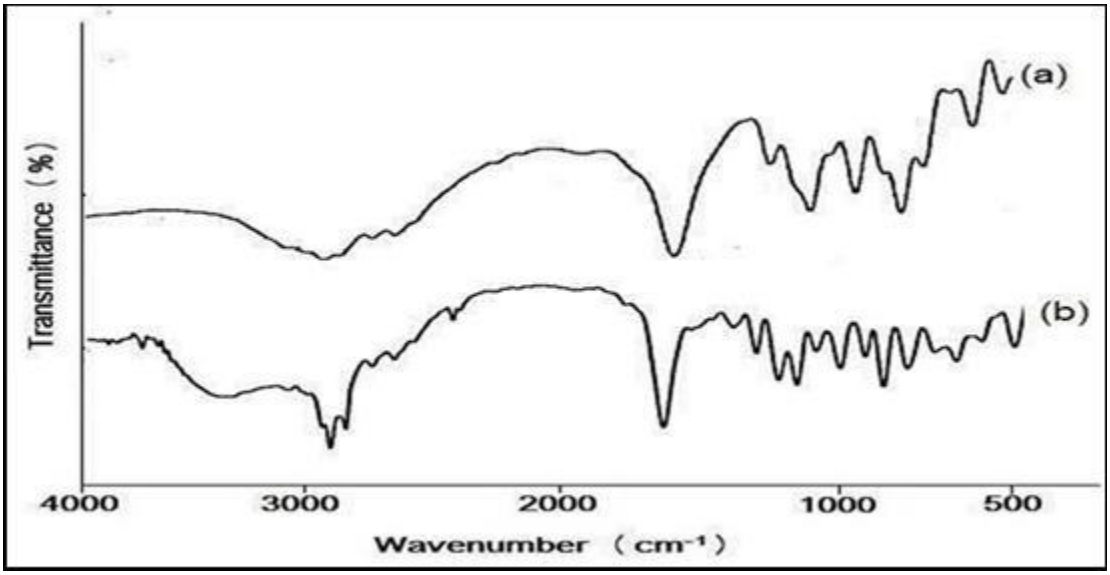

Fig. 7: Compatability study of IR spectrum in prepared Emulgel (a) and diclofenac sodium gel (b).

Conclusions

Emulgels are the one which combine gels and emulsions together. In this study, topical emulgel of *Crotolaria juncea* was formulated and subjected to physiochemical studies i.e. rheological studies, spreadability, in vitro drug releases study and showed prominent result. In vitro drug release behavior of drug from formulation. From the observed results that was observed that Emulgel containing 2% of concentration of *Crotalaria juncea* extract formulation gel F4 shows better result when compared to F1 to F5 formulation which produce better consistency and spredability. The compatibility studies of formulation gel F4 with diclofenac sodium gel also gives good result. The F4 gel forms water washable because of its water solubility and has wider prospects to be used as tropical drug delivery system. The successfully developed F4 gel showed good homogeneity, suitable pH, no skin irritation and good stability. The maximum percentage of drug release was found to be 83.56% in 6 hrs in formulation F4. The drug permeation from optimized formulation i.e F4 was slow and steady. So finally it was concluded that there is increase in the drug release with respect to time.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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