

FORMULATION DEVELOPMENT AND STANDARDISATION OF HERBAL GEL CONTAINING METHANOLIC EXTRACT OF *BUTEA FRONDOSA*

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ABSTRACT

The present research has been undertaken with the aim to formulate and evaluate the herbal gel containing *Butea frondosa* extract. The gel formulation was designed by using methanol extract of *Butea frondosa* stem barks in concentration (5%) and evaluated using physiological measurements. The gel was prepared by using accurately weighted amount of drug along with other additives were poured into the fixed amount of hydrated Carbopol-934 dispersion with constant stirring. Finally the required amount of 0.5M sodium hydroxide solution was added to induce gelation. All the prepared gel formulations were subjected for preliminary evaluation such as pH, Viscosity and Rheological studies, Spreadability, Drug content uniformity, Skin irritation test, *In vitro* diffusion study, *In vitro* permeation studies and Drug Polymer Compatibility Studies. The optimized herbal gel formulation of the drug was subjected to accelerated stability studies at both 4°C and 37°C for about 3 months. A suitable UV method was developed for herbal gel formulation by using Phosphate buffer 6.8 as solvent and λ_{max} found to be 274 nm. The pH of all the formulations was in the range of 6.59 to 7.41, which lies in the normal pH range of the skin. The drug content was in the range of 96.4 to 99.6 %. The formulations did not produce any skin irritation, i.e., erythema and edema for about a week, when applied over the skin. The drug interaction FT-IR studies indicated that there was no chemical interaction between the drug and the polymers used in gel formulations.

Key Words: *Butea frondosa*, Methanolic extract, Herbal gel formulations, Carbopol 934, pH, Phosphate buffer.

INTRODUCTION

India has rich tradition of plant based knowledge of healthcare. The use of the plant based medication is gradually becoming popular throughout the world¹. Approximately, half of the world's twenty five best selling pharmaceutical agents are derived from natural products². *Butea frondosa* belongs to family-Fabaceae is a deciduous tree with a somewhat crooked trunk, up to 15m. in height and 1.6-2.0m (some times up to 3.80m) in girth³. The plant occurs and distribute commonly throughout the greater parts of India and Burma, up to an altitude of 3,000ft. and even higher in the outer Himalaya, Khandesh Akran upto 3,700ft., hills of South India up to 4,000 ft., Ceylon⁴.

The various parts used in the traditional medicine are gum, seeds, flowers, bark and leaves of the plant. The bark of *B. frondosa* is used as appetizer, aphrodisiac, laxative, anthelmintic, antidiarrhoeal, to cure ulcers and tumors^{4,6}. The stem bark, leaf and flower of *Butea frondosa* is used as an antimicrobial and anti-inflammatory agent⁷⁻¹⁰. The reported phyto constituents present in the *Butea frondosa* are butin and butrin¹¹, flavonoid compounds¹²⁻¹⁴, tannin¹⁵, butolic acid, shellolic acid and jalaric acid (sesquiterpene)¹⁶ and palasonin a monoterpene compound¹⁷.

Before the extract subjected for the formulation preparation, the chloroform and methanol extracts of *Butea frondosa* stem barks were evaluated for their analgesic and anti-inflammatory activity studies by using different animal models like hot plate method, tail immersion method, acetic acid method, Carrageenan induced rat paw edema method, Complete Freund's Adjuvant (CFA) method and compared with the Standard drugs i.e. Morphine sulphate (5mg/kg), Diclofenac Sodium (5mg/kg) and Indomethacin (4mg/kg) respectively. Among the two extracts the methanol extract at the dose of 200mg/kg body weight showed significant biological activities as compared to standard drug. The dose of the extract was selected for biological activity based on acute toxicity studies¹⁸. The experimental protocols were cleared by Institutional Animal Ethical Committee, Royal College of Pharmacy and Health Sciences, Berhampur (Vide No.10/2008/CPCSEA, dt.20.03.2008). Based on the analgesic and anti-inflammatory activities results, in the present study we design to formulate and evaluate the herbal gel formulations containing methanol extract of *B. frondosa* stem barks.

MATERIALS AND METHODS

Plant material

The stem barks of *Butea frondosa* were collected from the forest of Similipal Biosphere Reserve, Mayurbhanj, Orissa in August 2006. The plant material was identified and authenticated taxonomically at the Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah-711103, West Bengal, India (Ref no-CNH/I-I(59)/2006/Tech II, dated- 27.10.2006). A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Preparation of extracts

The collected stem barks were cleaned, dried under shade and powdered by a mechanical grinder. Hundred grams of the pulverized stem bark was extracted with petroleum ether, chloroform and methanol successively in a soxhlet apparatus. Petroleum ether was used in initial step of extraction for defatting the plant materials. The successive extracts were separately filtered and concentrated at reduced temperature on a rotary evaporator. The yield was found to be around 2.11; 4.38 and 18.08% (W/W) respectively. The biologically potent methanol extract was prepared for herbal gel formulation.

Preparation of Herbal gel

The required quantity of Carbopol-934 was slowly sprinkled into purified water I.P. with constant stirring to get the uniform dispersion and then kept overnight for hydration. The accurately weighted amounts of drug along with other additives were poured into the fixed amount of hydrated Carbopol-934 dispersion with constant stirring. Finally the required amount of 0.5M sodium hydroxide solution was added to induce gelation. The composition of herbal gel prepared from Methanolic extract of *Butea frondosa* is tabulated in Table 1

Evaluation of Herbal gel

All the prepared gel formulations were subjected for preliminary evaluation as follows¹⁹⁻²¹:

pH

The pH of various gel formulations were determined by using digital pH meter. 2.5gm of gel was accurately weighed and dispersed in 25ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and the average

values are represented in Table 2. The pH of dispersions was measured using pH meter (Systronics digital-DI-707).

Viscosity and Rheological studies

Viscosities of gel 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 settings from 10rpm to 100rpm with 30seconds between 2 successive speeds and then in a descending orders.

Spreadability

Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin or affected part. A special apparatus as suggested by Panigrahi *et al*, (2006) has been designed to the spreadability of gel formulations. The spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability.

Spreadability is calculated by using the formula:

$$S = m/t$$

Where,

m = weight tide to upper slide

l = length moved on the glass slide

t = time taken to separate the slides completely from each other.

In this present experiment, m = 250 gm, l = 3.8cm, 'S' is recorded in Table 2.

Drug content uniformity

About 1 gm of gel was accurately weighed and transferred to 100ml volumetric flask to which about 70ml of methanol was added. After mixing, the volume was made up to 100ml with methanol. The content was filtered through a suitable filter paper. An aliquot of 1ml was pipetted out from the filtrate and suitably diluted with methanol. Then the extract was estimated spectrophotometrically by using Shimadzu UV/VIS spectrophotometer-1700 at respective λ max.

Skin irritation test

The test was performed on albino mice for the prepared gels. Albino mice weighing about 25-30gm were taken for the test. The animals were divided into two groups, viz. control and test, each containing seven animals. The gel containing the extract was used on the test animals. A piece of cotton wool soaked in saturated drug solution was placed on the back of the albino mice taken as control. The gel and cotton wools were secured firmly with the help of adhesive tapes. Aqueous solution of 0.8% formalin was applied as a standard irritant. The animals were observed for seven days for any sign of edema and erythema.

In vitro diffusion study

Dialysis membrane-50 (Av. Flat width- 24.26mm, Av. Diameter-14.3mm) obtained from Hi-media laboratories Pvt Ltd. was used for this study. In modified Kiescary Chien diffusion cell, 2gm of gel was kept in donor compartment. The entire surface of membrane was in contact with the receptor compartment containing 60ml of phosphate buffer pH 6.8. The receptor compartment was continuously stirred (100rpm) using a magnetic stirrer. The temperature maintained was 37±1°C. The study was carried out for 8hr with the interval of 0.5, 1, 2, 3, 4, 5, 6, 7 and 8hr. The surface area available for diffusion was calculated and was found to be 3.14cm². The sample was withdrawn at predetermined time interval and same volume was replaced with fresh phosphate buffer. The absorbance of withdrawn sample was measured after suitable dilution at respective λ max to estimate drug concentration. The experiment was carried out in triplicate and average values are reported.

In vitro permeation studies

Kiescary Chien diffusion cell mounted with hairless rat skin was used for drug permeation study. 2gm of gel was taken into the cell

(donor compartment) and phosphate buffer pH 6.8 in receptor compartment which is agitated using magnetic stirrer (100rpm) and temperature maintained to 37±1°C was maintained. The sample was withdrawn at predetermined time intervals and same volume replaced with fresh buffer medium. Absorbance was measured after suitable dilution at respective λ max to estimate drug concentration.

Drug Polymer Compatibility Studies

The interaction studies were carried out to ascertain any kind of chemical interaction of drug with the excipients used in the preparation of gel formulations. Fourier-transform infrared (DRS) spectra were obtained by using an FT IR-Affinity-1 spectrophotometer (DRS-8000) SHIMADZU, Japan. The dried pure drug sample BFP was previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:5 (Sample: KBr) ratio, respectively. The KBr powder was used as blank for background correction in FT-IR (DRS) studies. Forty five scans were obtained at a resolution of 4 cm⁻¹, from 4000 to 300 cm⁻¹.

Stability Studies of optimized herbal gel formulations

The optimized gel formulation of the drug was subjected to accelerated stability studies at both 4°C and 37°C for about 3 months. The gel was observed after each week for possible changes in color, odour, consistency, phase separation, pH, viscosity and spreadability.

Development of UV-VIS Spectrophotometric method for estimation of formulated Herbal gel

Scanning and determination of maximum wavelength (λ max)

In order to ascertain the wavelength of maximum absorption of the extract, different concentrations of the extract (10 µg/ml, 20µg/ml and 30µg/ml) in phosphate buffer pH 6.8 were scanned using spectrophotometer within the wavelength range of 400-200 nm against phosphate buffer pH 6.8 as blank and the wavelength corresponding to maximum absorbance was noted.

Preparation of standard stock solution

Accurately weighed 100mg of extract was dissolved in 3ml of methanol in 100ml volumetric flask and volume was made up to the mark with phosphate buffer pH 6.8 to give a clear solution of 1000µg/ml concentration.

Preparation of working standard solutions and construction of Calibration Curve

A series of different concentrations of extract solutions were prepared from working stock solution. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6.....1.8, 1.9 and 2.0 ml solutions were pipetted out from working stock solution and were transferred in to 10 ml volumetric flasks. 10,20,30,40 up to 200µg/ml solutions were obtained respectively on making up the solution to 10 ml with phosphate buffer pH 6.8. The absorbances of all these solutions were measured against a blank at respective λ max using a UV double beam spectrophotometer (UV/Vis-1700, Shimadzu, Japan). A standard plot of absorbance v/s concentration of extract gives the standard calibration curve of the extract. This curve was used to determine in vitro drug release and drug content of herbal gels and the observations are shown in Table 3.

RESULTS AND DISCUSSION

The various physicochemical properties of the prepared gel formulations are shown in Table 2. From the results it is clearly evident that all the gel formulations showed good gelling property and homogeneity. The pH of all the formulations was in the range of 6.59 to 7.41, which lies in the normal pH range of the skin. The drug content was in the range of 96.4 to 99.6 %. The formulations did not produce any skin irritation, i.e., erythema and edema for about a week, when applied over the skin. The rheological behaviors of the gel formulations were studied with Brookfield viscometer. The results indicated that as torque increased, shear stress increased and viscosity decreased. A comparative study of viscosity and

spreadability showed that with increase in viscosity of the formulation, the spreadability decreased and vice versa. The absorption curve of *Butea frondosa* methanolic extract showed characteristic absorption maximum at 274 nm in 6.8 Phosphate buffer. The drug obeyed Beer's law in the concentration range of 10mcg/ml to 180mcg/ml, and it was found to be linear with $r^2 = 0.999$, regression equation $Y = 0.017x + 0.003$. The FT-IR spectra of gel formulation did not show the presence of any additional peaks for new functional groups. The major peaks of the drug remained unchanged in the mixture. These results suggest absence of any chemical interaction between the drug BFP and the polymers used in gel formulation. Hence, the drug was found to be compatible with all the excipients used in the formulations. Among all the gel formulations studied, the formulation BFG-2 showed good in-vitro drug release in diffusion and permeation studies after 8 hours was found to be 92.37% and 98.29% respectively. From the accelerated stability studies, formulation BFG-2 showed no changes in colour, odour, consistency, spreadability, pH and phase separation after storing at different conditions for about 3 months. Therefore the formulation BFG-2 was optimized.

CONCLUSION

As per the reported traditional uses of the plant for different topical applications, the present research work was carried out to develop a new topical herbal gel formulation. The prepared herbal gel was further evaluated for pH, Viscosity and Rheological studies, Spreadability, Drug content uniformity, Skin irritation test, In vitro diffusion study, In vitro permeation studies and Drug Polymer Compatibility Studies. The incorporation of DMSO to the formulation, which enhance the diffusion and permeation of the drug. The optimized formulation BFG-2 complies all the parameters. Further studies on in vitro models are required to evaluate the biological potency of the prepared herbal gel formulation and then it can be useful for the clinical application.

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Table 1: Composition of various gel formulations containing *Butea frondosa* Methanolic extract

Ingredients (% w/w)	BFG-1	BFG-2	BFG-3	BFG-4
Extract	5	5	5	5
DMSO (%v/w)	-	6	6	6
Carbopol 934	0.5	0.5	1.0	1.5
Methyl Paraben	0.15	0.15	0.15	0.15
Propyl Paraben	0.5	0.5	0.5	0.5
Purified Water (qs)	100ml	100ml	100ml	100ml

BFG = *Butea frondosa* Methanolic extract gel formulation

Table 2: Characteristics of herbal gel formulation

Formulation Code	pH	Viscosity (cps)	Spreadability (g.cm/s)	Drug content (%w/w)#	Skin irritation
BFG-1	6.67	1540	14.52	96.7±0.12	-
BFG-2	6.59	1542	14.55	99.6±0.27	-
BFG-3	7.02	1655	13.09	97.3±0.35	-
BFG-4	7.41	1870	12.05	96.4±0.26	-

#values expressed as Mean ±SD, n=3 and '-' indicates no skin irritation

Table 3: Percentage drug release (Diffusion study) for formulation BFG

Time (hr)	% Cumulative drug release*			
	BFG-1	BFG-2	BFG-3	BFG-4
0.5	1.93±0.99	15.41±0.81	13.16±0.94	10.12±1.02
1	7.79±1.02	24.20±2.87	20.31±2.81	19.23±2.06
2	15.98±2.4	36.12±1.79	34.17±3.02	33.31±2.36
3	26.17±1.91	49.33±1.56	43.27±2.42	46.51±2.18
4	31.81±1.21	61.53±1.95	54.16±2.87	55.10±2.3
5	44.90±2.07	72.27±2.34	65.21±1.85	62.45±2.26
6	53.67±2.3	88.18 ±2.95	74.42±2.41	70.13±2.92
7	60.51±1.92	91.58±2.13	85.43±2.20	72.01±2.01
8	61.11±1.45	92.37±2.71	86.54±1.98	72.53±2.37

BFG = *Butea frondosa* Methanolic extract gel formulation, * values expressed as Mean cumulative percent±S.D. (n=6)

Table 4: Percentage drug release (Permeation study) for formulation BFG

Time (hr)	% Cumulative drug release*			
	BFG-1	BFG-2	BFG-3	BFG-4
0.5	5.63±0.91	20.54±0.81	19.81±0.91	17.72±0.86
1	11.77±1.03	25.92±1.78	24.03±1.73	22.00±1.33
2	21.15±1.27	41.62±1.65	37.31±1.43	35.50±1.85
3	31.73±1.87	56.88±1.78	50.27±1.9	49.46±1.75
4	37.88±1.3	69.01±1.55	61.09±1.23	58.21±1.22
5	51.79±2.13	81.07±2.44	72.72±1.85	69.24±2.07
6	65.67±1.9	93.89±2.93	79.14±2.08	72.25±1.98
7	67.15±1.77	97.58±2.13	91.71±2.17	87.32±1.66
8	68.75±1.66	98.29±2.21	93.63±2.04	89.19±2.62

BFG = *Butea frondosa* Methanolic extract gel formulation, * values expressed as Mean cumulative percent±S.D. (n=6)

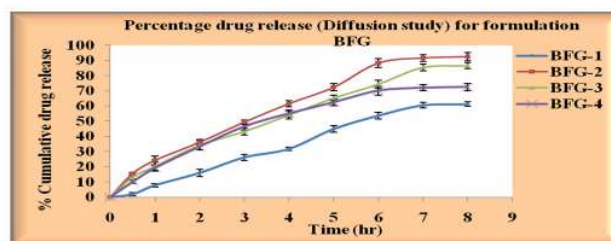


Fig. 1 Diffusion profile of *Butea frondosa* extract from various gel formulations

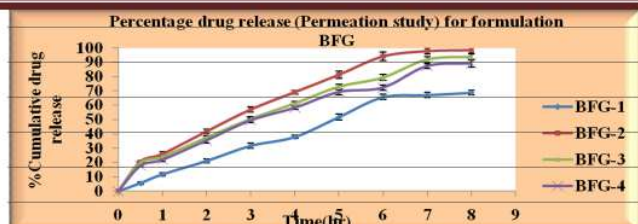


Fig-2 Permeation profile of *Butea frondosa* extract from various gel formulations

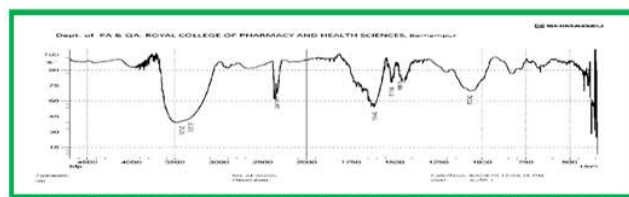


Fig. 4 FT-IR Spectrum of BFP

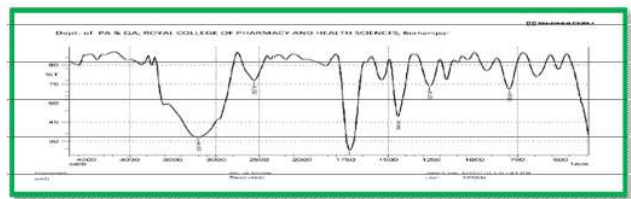


Fig. 3 FT-IR Spectrum of Carbopol

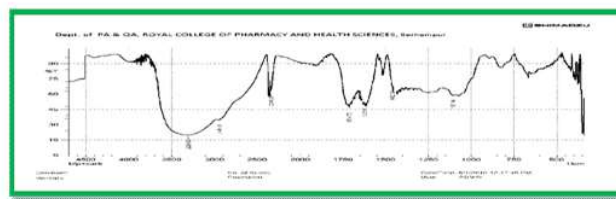


Fig. 5 FT- IR Spectrum of BFP + Carbopol

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