AMERICAN JOURNAL OF PHARMTECH RESEARCH

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FORMULATION AND STANDARDISATION OF HERBAL GEL CONTAINING METHANOLIC EXTRACT OF *CALOPHYLLUM INOPHYLLUM.*

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ABSTRACT

The present research has been undertaken with the aim to formulate and evaluate the herbal gel containing *Calophyllum inophyllum* extract. The gel formulation was designed by using methanol extract of *Calophyllum inophyllum* 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, Drug Polymer Compatibility Studies. The optimized herbal gel formulation of the drug was subjected to accelerated stability studies at both 4^0C and 37^0C 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 284 nm. The pH of all the formulations was in the range of 6.63 to 7.35, which lies in the normal pH range of the skin. The drug content was in the range of 96.6 to 99.5 %. 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: *Calophyllum inophyllum*, Methanolic extract, Herbal gel formulations, Carbopol 934, pH, Phosphate buffer.

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Received 21 October 2011, Accepted 14 November 2011

Please cite this article in press as: Mishra US *et al.*, Formulation And Standardisation of Herbal Gel Containing Methanolic Extract of *Calophyllum Inophyllum.* American Journal of PharmTech Research 2011.

INTRODUCTION

India has rich tradition of plant based knowledge of healthcare. The use of the plant based medication is gradually becoming popular throughout the world[1]. Approximately, half of the world's twenty five best selling pharmaceutical agents are derived from natural products[2]. *Calophyllum inophyllum* belongs to family Clusiaceae (syn. Guttiferae) is a medium sized to large evergreen tree that average 8-20m in height with a broad spreading crown of irregular branches[3]. In India it is distributed in the coastal regions of Maharashtra, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh and Orissa. About seven species occur in India. Some species are ornamental and others yield timber, commercially classified as POON and oil[4].

In different parts of India, the plant is known by different vernacular names /local names are English - Alexandra Laurel, Alexandrian Laurel, Hindi - Sultanachampa, surpunka, undi, Sanskrit - Nagachampa, punnaga, surangi, Oriya - Polang, ponnang, Tamil- Pinnai, punnagam[3,4].

As per the ethnomedicinal information the various parts of *Calophyllum inophyllum* possess medicinal properties. The fresh bark of *C. inophyllum* is used to treat diabetes[5]. The fresh fruit and its oil used externally against rheumatism, in topical infection and seborrhea in human adult[6,7]. The dried leaf and its decoction used to cure rheumatism , skin-infections[8], cuts and sores[9-11].The fresh leaves infusion are used to cure bacterial infection, fungal infection and as vermifuge/ pediculicide[7].The resin are used orally as an emetic and purgative[12]. Dried seed extract are used against rheumatism in human adult[13]. In Java the trees are supposed to possess diuretic properties[3, 4]. The stem barks possess antibacterial and analgesic activities[14].

The reported chemical constituents present in *C. inophyllum* are flavonoid compound Amentoflavone[15,16], Steroid compound campesterol[17,18], Arachidic acid lipid[18], xanthone derivative Brasilixanthone- B and Buchanaxanthone[19,20], coumarin derivatives Calocoumarin-A, Calocoumarin-B, Calocoumarin-C and Apetalolide[21,22] , and Beta Amyrin a triterpene[23] .

Before the extract subjected for the formulation preparation, the chloroform and methanol extracts of *Calophyllum inophyllum* 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 studies[24]. 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 *Calophyllum inophyllum* stem barks.

MATERIAL AND METHOD

The stem barks of *Calophyllum inophyllum* 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 2.52; 4.81 and 21.36% (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 *Calophyllum inophyllum* is tabulated in Table 1.

Evaluation of herbal gel[25-27]

All the prepared gel formulations were subjected for preliminary evaluation as follows:

Table 1: Composition of various gel formulations containing *Calophyllum inophyllum* extract

Ingredients (% w/w)	CIG-1	CIG-2	CIG-3	CIG-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

CIG = *Calophyllum inophyllum* Methanolic extract gel formulation

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).

Table 2: Characteristics of various herbal gel formulations

Formulation Code	pH	Viscosity(cps)	Spreadability (g.cm/s)	Drug content (%w/w)	Skin irritation
CIG-1	6.75	1522	14.59	99.1±0.72	-
CIG-2	6.63	1525	14.61	99.5±0.63	-
CIG-3	7.31	1662	13.88	97.8±0.40	-
CIG-4	7.35	1892	13.03	96.6±0.51	-

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

Viscosity and Rheological studies

Viscosities of gels were determined using Brookfield viscometer. Gels were tested for their rheological characteristics at 25^0C 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=ml/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 pipette 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 in to 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 ± 1^0C. 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 3.14cm^2. 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 Table 3 and Figure 1 respectively.

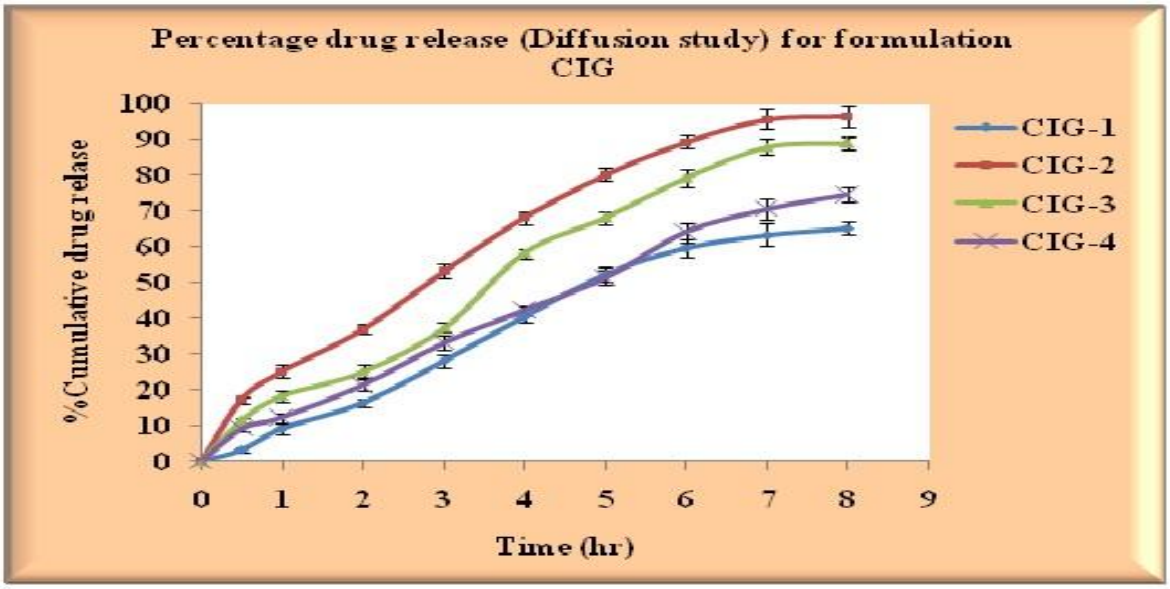

Figure 1:Diffusion profile of *Calophyllum inophyllum* extract from various gel formulations

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

Time (hr)	% Cumulative drug release*			
	CIG -1	CIG -2	CIG -3	CIG -4
0.5	3.13±0.79	17.16±0.93	11.26±0.96	9.22±0.89
1	9.09±1.66	25.21±1.75	18.34±1.66	12.11±1.51
2	16.38±1.32	37.11±1.39	25.16±1.85	21.31±1.57
3	28.17±1.76	53.25±1.89	37.54±1.46	33.25±1.88
4	40.33±1.44	68.10±1.77	58.24±1.34	42.16±1.49
5	52.39±1.93	80.34±1.88	68.10±1.86	51.37±2.09
6	59.71±2.37	89.42±1.94	79.25±2.47	64.10±2.7
7	63.53±2.99	95.77±2.62	88.10±2.11	70.64±2.91
8	65.35±1.63	96.66±2.87	89.12±1.97	74.55±2.04

CIG = *Calophyllum inophyllum* Methanolic extract gel formulation, * values expressed as Mean cumulative percent±S.D. (n=6).

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 ± 1^0C 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 and mentioned in Table 4 and Figure 2.

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

Time (hr)	% Cumulative drug release*			
	CIG -1	CIG -2	CIG -3	CIG -4
0.5	4.93±0.89	21.93±0.81	20.58±0.89	16.99±0.68
1	12.54±1.51	26.73±1.07	25.10±1.54	22.00±1.05
2	23.77±2.42	42.37±1.79	38.00±1.31	34.95±1.44
3	32.91±2.64	58.08±1.72	49.72±1.93	52.22±1.85
4	37.00±2.9	70.05±2.46	62.13±1.66	60.62±1.79
5	49.94±2.07	82.31±2.34	74.07±1.53	67.92±2.88
6	64.55±2.3	94.99 ±2.93	81.97±2.33	74.00±1.97
7	67.90±1.12	98.03±2.77	93.21±2.93	87.06±2.83
8	70.97±1.07	99.91±1.33	96.44±1.94	87.73±1.17

CIG = *Calophyllum inophyllum* Methanolic extract gel formulation, * values expressed as Mean cumulative percent±S.D. (n=6)**.**

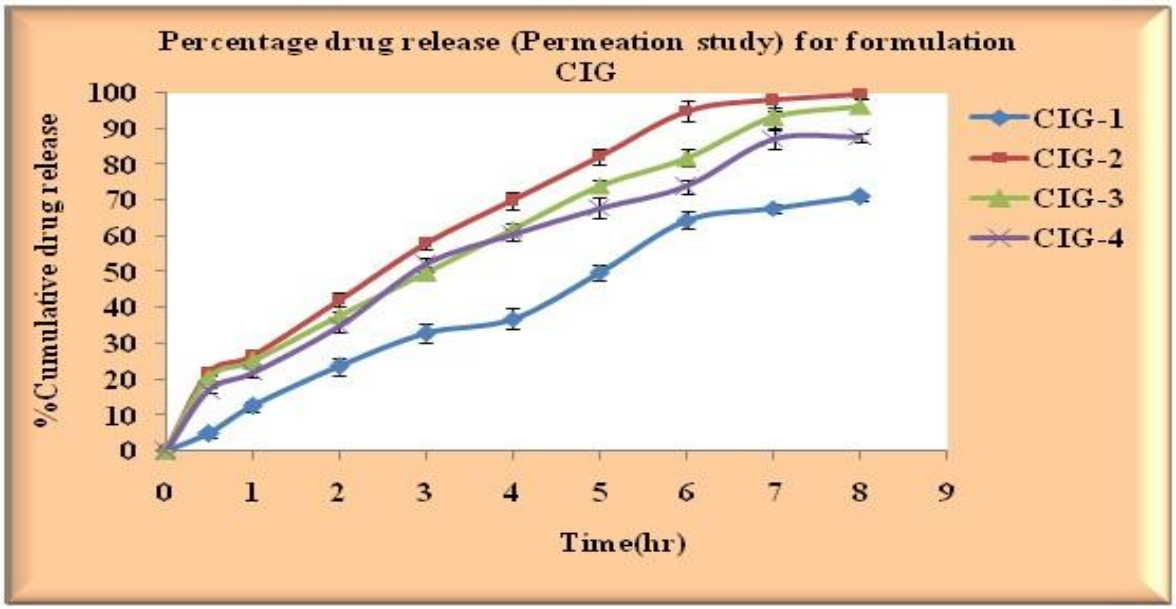

Figure 2: Permeation profile of *Calophyllum inophyllum* extract from various gel formulations

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 FTIR-Affinity-1 spectrophotometer (DRS-8000) SHIMADZU, Japan. The dried pure drug sample CIP 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 formulation

The optimized gel formulation of the drug was subjected to accelerated stability studies at both 4^0C and 37^0C for about 3 months. The gels were 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 (λmax)

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 (Figure 3).

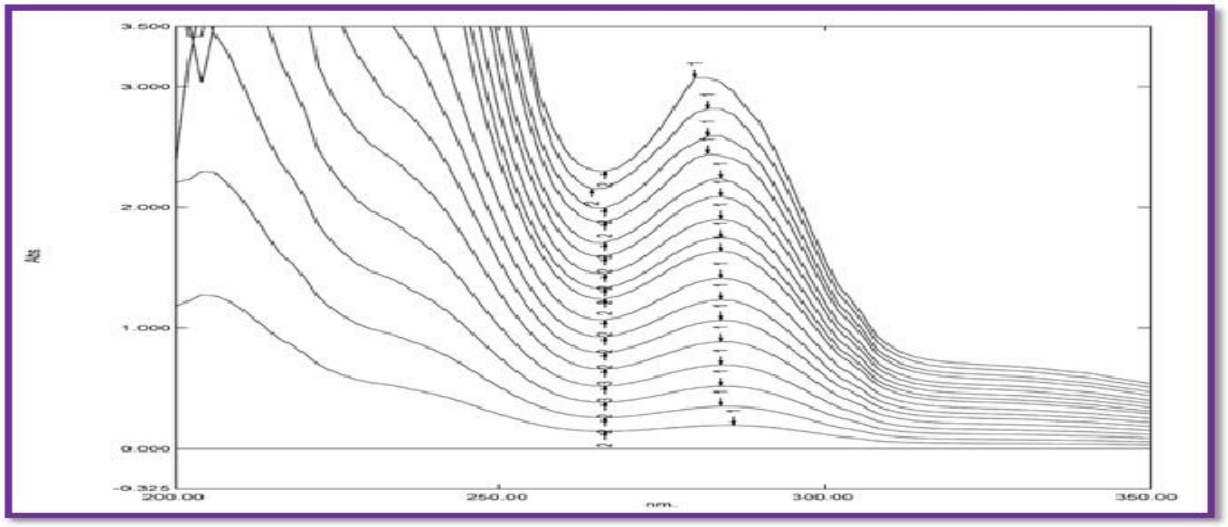

Figure 3: Overlay spectra of *Calophyllum inophyllum* Methanolic extract in 6.8 phosphate buffer at λ_{max} of 284nm

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 pipette 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 λmax 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 (Figure 4). This curve was used to determine in vitro drug release and drug content of herbal gels and the observations are shown in Table 5.

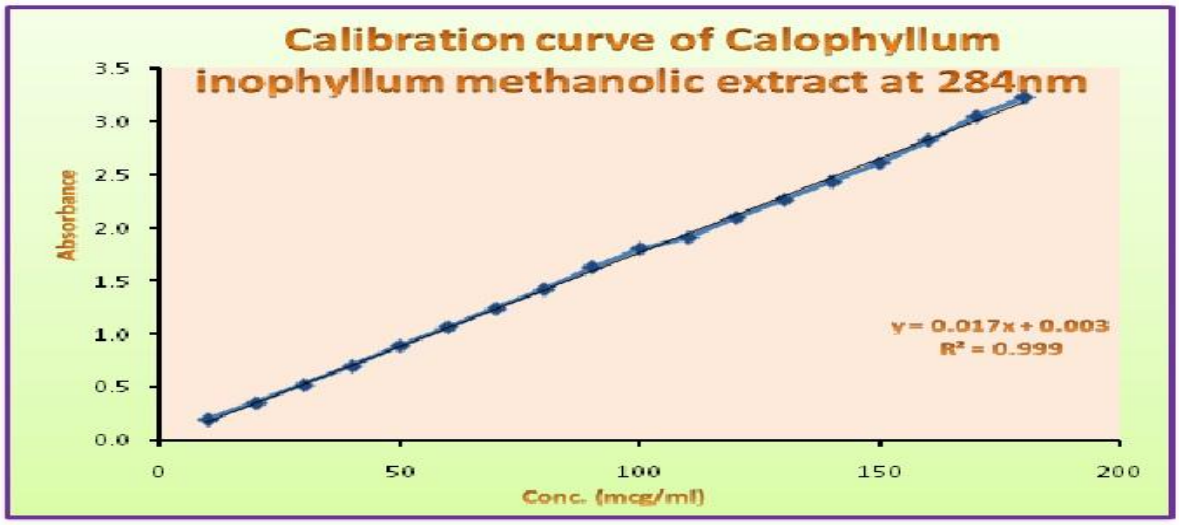

Figure 4: Calibration curve of *Calophyllum inophyllum* Methanolic Extract at 284nm

Table 5: Calibration of *Calophyllum inophyllum* Methanolic extract in 6.8 phosphate buffer at λ_{max} of 284nm

Sl. No.	Conc. (mcg/ml)	Absorbance at 284 nm
1	10	0.197
2	20	0.353
3	30	0.52
4	40	0.698
5	50	0.889
6	60	1.067
7	70	1.235
8	80	1.417
9	90	1.625
10	100	1.801
11	110	1.904
12	120	2.091
13	130	2.258
14	140	2.429
15	150	2.604
16	160	2.817
17	170	3.039
18	180	3.218

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 6.63 to 7.35, which lies in the normal pH range of the skin. The drug content was in the range of 96.6 to 99.5 %. 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 *Calophyllum inophyllum* Methanolic extract showed characteristic absorption maximum at 284 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 formulations did not show the presence of any additional peaks for new functional groups. The major peaks of the drug remained unchanged in the mixture were observed in FT-IR spectra (Figure 5-7).

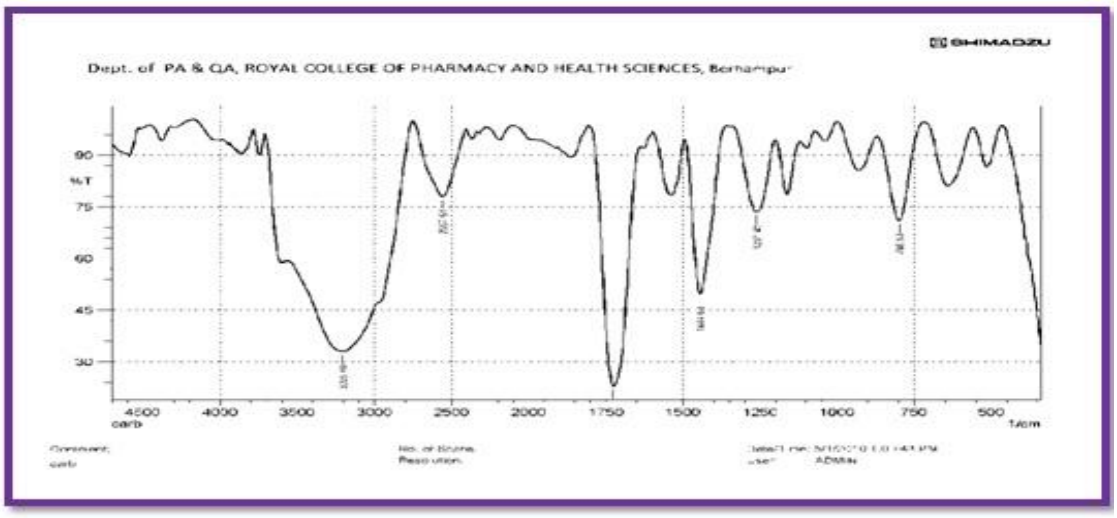

Figure 5: FT-IR Spectrum of Carbopol

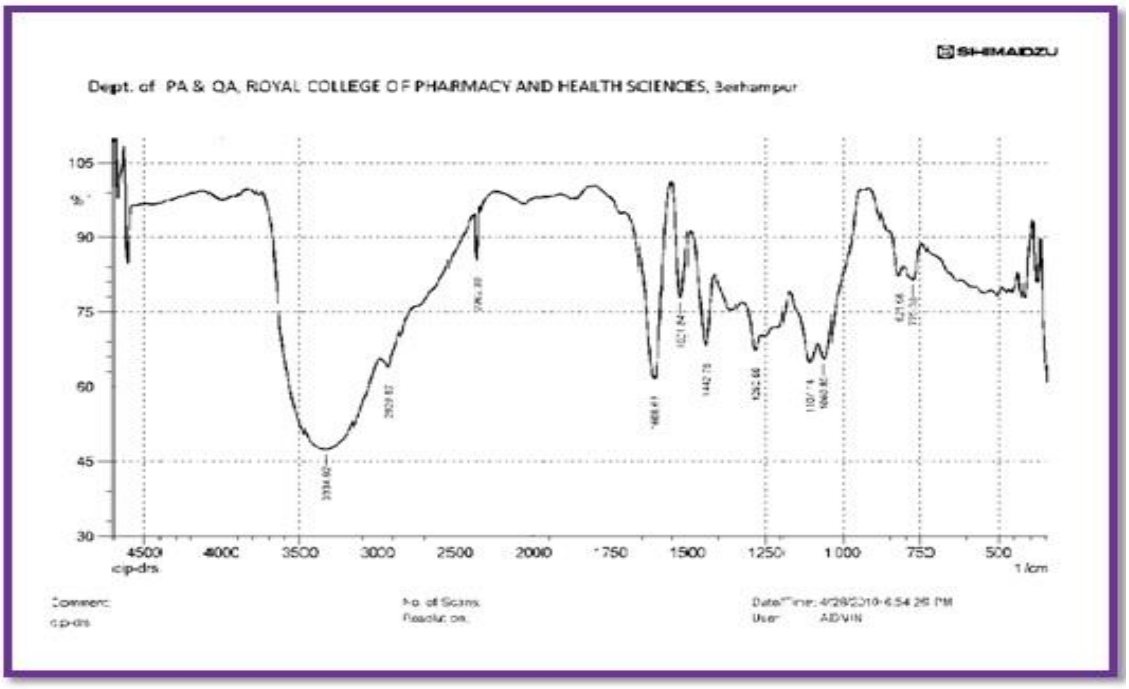

Figure 6: FT-IR Spectrum of CIP

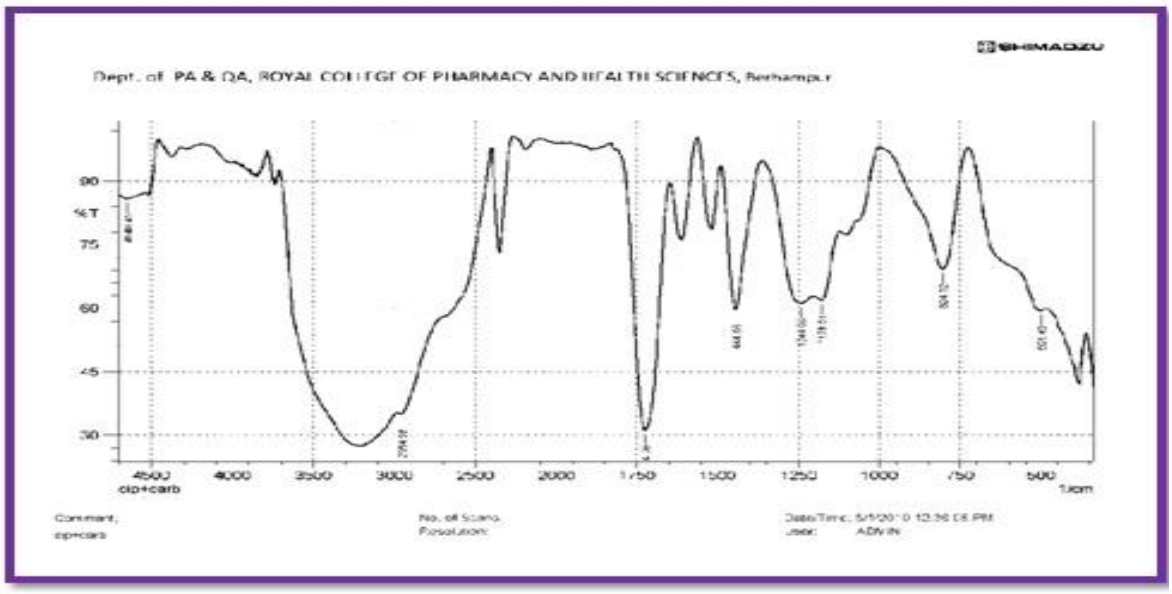

Figure 7: FT-IR Spectrum of CIP + Carbopol

These results suggest absence of any chemical interaction between the drug CIP and the polymers used in gel formulations. Hence, the drug was found to be compatible with all the excipients used in the formulations. Among all the gel formulations studied, the formulation CIG-2 showed good in-vitro drug release in diffusion and permeation studies after 8 hours was found to be 96.66 and 99.91% respectively. From the accelerated stability studies, formulation CIG-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 CIG-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 CIG-2 complies all the parameters.

Further studies on in vitro models are required to evaluate the potential of the herbal gel formulation and then it can be useful for the clinical application.

ACKNOWLEDGEMENTS

The authors thanks to Management, Royal College of Pharmacy & Health Sciences, Berhampur, Orissa, India for providing required facilities to carry out this research work.

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