

**FORMULATION AND EVALUATION OF HERBAL GEL OF
ACALYPHA INDICA**

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ABSTRACT

The aim of the present study is to fabricate the *Acalypha indica* extract as herbal gel. FTIR studies confirmed that the formation of complex. Totally seven formulations were fabricated by using Carbopol 934 as base with dried extract of *Acalypha indica*. All formulations were evaluated for pH, spreadability, extrudability, drug content, percentage yield, entrapment efficiency, *in-vitro* diffusion study. Formulation 4 (F4) was selected by the *in-vitro* diffusion study, which showed maximum release at 8hrs. The selected formulation 4 (F4) showed anti-bacterial activity against *S.aureus*. The stability study was conducted and it's showed that there was no significant change in

homogeneity, drug content, pH, spreadability, extrudability, viscosity and percentage cumulative drug release.

KEYWORDS: *Acalypha indica*, Carbopol 934, herbal gel, Anti-bacterial activity.

INTRODUCTION

Acalypha indica consists of 450 species and these are used for various medicinal uses like stomachache, dyspepsia, dermatitis and eye infections.^[1,2,3] Several biological values also there such as wound healing, anti-inflammatory, anti-oxidant and anticancer activities, especially for the cure of scabies and skin infections.^[4,5,6,7]

Most of plant based medicaments are popular in pharmaceutical agents. The leaves of *Acalypha Indica* Linn were previously investigated to have antifungal activity.^[8,9,10,11]

Among the semisolid dosage forms, gels are playing important role because of less greasy and easily absorption.^[12,13,14,15,16]

In this study, the ethanolic extract of *Acalypha indica* is showed the antibacterial effect and they are formulated as herbal gel by adding methyl paraben, propyl paraben and Carbopol, with dried extract of *Acalypha indica*. From the study it can be calculated that the fabricated gel may be used for antimicrobial activity.^[17,18,19,20,21,22]

MATERIALS

Dried leaves of *Acalypha indica* authenticated by botanical survey of India, Coimbatore. The leaves were collected at local area of Telungupalayam. All the chemicals used were in analytical grade.

METHODS

Preparation of plant extract

The leaves were washed and dried then extracted with ethanol using Soxhlet apparatus. The solvent was removed and concentrated using water bath and prepared extracts were stored for further studies.^[14,15]

Determination of λ Max

The stock solution was prepared by dissolving 10ml of *Acalypha indica* extract in 100ml of pH 7.2 phosphate buffer. From this solution, 10ml was taken and diluted to 100ml with the buffer solution. Then the prepared solution was scanned in a wavelength at 265nm using UV spectrophotometer.

Determination of calibration curve

The diluted stock solution was taken in the range of 5-25 μ g/ml in 100ml volumetric flask; the final volume was made by using pH 7.2 phosphate buffer solution. The diluted solutions absorbances were measured at 265nm using UV spectrophotometer.

Preparation of herbal gel procedure

Accurately weighed quantity of carbopol 934, was soaked with water and this is used as base. The dry extract of *Acalypha indica* was mixed thoroughly with the base. To this base glycerin and preservatives are added and homogenized further. Different formulations were developed with different concentration of ingredients and the composition was shown in **Table no.1**

Table no. 1: Composition of herbal gel formulation of *Acalypha indica*.

Excipients	F1	F2	F3	F4	F5	F6	F7
Methyl paraben IP	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g
Propyl paraben IP	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g	0.5g
Carbopol 934p	1.5g	1.2g	1.35g	1.5g	1.2g	1.35g	1.5g
Glycerin IP	27.5ml	22.8ml	21.65ml	17.5ml	22.8ml	21.65ml	17.5ml
Purified water	70ml	70ml	70ml	70ml	70ml	70ml	70ml
Dry extract of <i>Acalypha indica</i>	-	5g	6g	10g	5g	6g	10g
Total	100	100	100	100	100	100	100

**Figure no. 1: *Acalypha indica* herbal gel.****Evaluation of *Acalypha indica* herbal complex****Microscopic view**

Optical microscopy was used for known of the gel and the gel was observed at 10X magnification.

Fourier Transformer-Infra Red Spectroscopy (FTIR) Analysis

The FT-IR spectroscopy was used for analysis of drug, mixture of drug and excipients. The drug alone and mixture of drug and excipients was taken and IR spectra of drug in KBr pellets at moderate scanning speed between $4000\text{--}400\text{cm}^{-1}$ was carried out using FTIR.

Percentage practical yield

The formulated herbal gel was weighed accurately and the percentage practical yield was calculated by using the formula:^[14,15]

$$(\%) \text{ Yield} = \frac{(\text{Practical yield})}{(\text{Theoretical yield})} \times 100$$

Entrapment efficiency

100mg of *Acalypha indica* herbal gel were centrifuged at 2000rpm for 20 min, the free drug

in the supernatant was determined at 265nm using UV-Visible spectrophotometer by using the formula:^[14,15]

$$\text{Entrapment efficiency (\%)} = \frac{(\text{Total amount of drug}) - (\text{amount of free drug})}{\text{Total amount of drug}} \times 100$$

Drug content

Acalypha indica herbal gel equivalent to 10 mg of material was taken into a 100 ml volumetric flask and were dissolved in specified quantity of ethanol. Volume was adjusted and drug content was determined spectrophotometrically at 265nm using UV spectrophotometer.

In-vitro Drug diffusion study through egg membrane

The *in-vitro* diffusion study was carried out by using Franz Diffusion Cell. The egg membrane was mounted between the donor and receptor compartment. The receptor compartment contains 15 ml of pH 7.2 phosphate buffer and the temperature was maintained at 37°C and was constantly stirred. The samples were withdrawn at specific time intervals and they were analyzed at 265nm using UV spectrophotometer.

Anti-Bacterial activity

The optimized formulation was tested *in-vitro* for its antimicrobial activities against *P.aureus* by well diffusion assay method known antibiotic such as Gentamycin was used as a reference (as standard drug) against bacteria and inhibition zone of anti-bacterial activity was critically examined.^[21,22]

Scanning electron microscopy (SEM) analysis

Scanning electron microscopy study was done to determine the surface morphology, size and shape of prepared *Acalypha indica* herbal gel formulation.

Evaluation of herbal gel of *Acalypha indica*

Homogeneity

The optimized gel was tested for homogeneity by visual inspection. It was tested for their appearance and presence of any aggregates.

Measurement of pH

The pH of the herbal gel was measured with the help of digital pH meter. 0.5g of herbal gel

was dissolved in 50ml of distilled water and stored for 2hrs. The measurement of pH of each formulation was determined.

Rheological study

The measurements of viscosity of optimized gel were carried out with Brookfield Viscometer. The readings of formulation were taken.

Spreadability

On a glass plate of 10×5cm, 350mg gel was taken and another plate of same sized was dropped from a distance of 5cm. After 1 minute the diameter of the circle spread was measured.^[14,15]

Extrudability

The study of extrudability was determined by measuring the weight (in grams) required to extrude at least 0.5cm gel from lacquered aluminium collapsible tube in 10sec^[14,15]. Then the extrudability was calculated by using the following formula.

$$\text{Extrudability} = \frac{\text{Applied weight to extrude gel from tube (in gram)}}{\text{Area (in cm}^2\text{)}}$$

In-vitro drug release study

The *in-vitro* drug release studies were carried out using a modified Franz diffusion cell (FDC). The optimized formulation was placed between donor and receptor compartment. Phosphate buffer pH 7.2 was used as diffusion media. The temperature of the cell was maintained at 37°C and the solution was stirred continuously. 1ml of aliquots sample were withdrawn with the specific time interval for 8 hours and same quantity was replaced. The samples withdrawn were analysed spectrophotometrically at 265nm by using UV Spectrophotometer.

Stability studies

The stability studies were conducted for the optimized formulation as per ICH guidelines. The sample was withdrawn at initial, 30th and 45th day and to analyze the physical appearance, drug content and percentage cumulative drug release.^[14,15]

RESULTS AND DISCUSSION

Determination of λ_{max}

The *Acalypha indica* extract absorption spectrum was scanned between 400-4000nm in phosphate buffer. The peak was shown in **Figure no.2**.

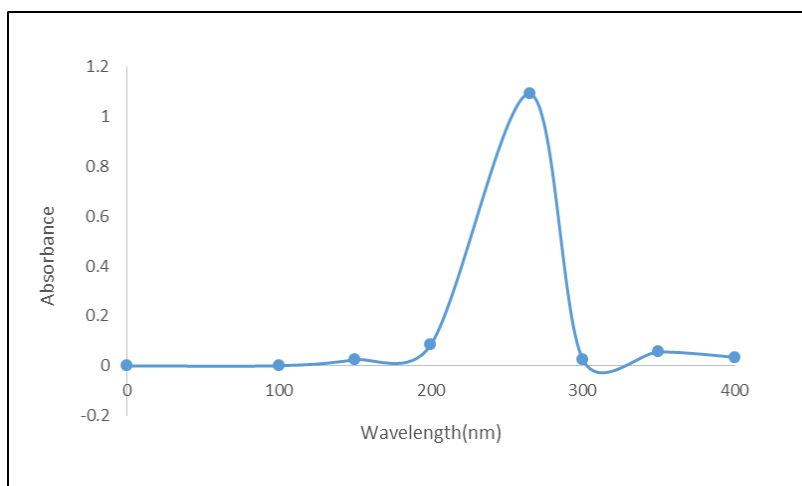


Figure no. 2: Absorption spectrum of *Acalypha indica* extract.

Calibration curve of *Acalypha indica* extract

The λ_{max} *Acalypha indica* of extract was determined by scanning the prepared solution in the wavelength range of 200-400 nm. The maximum wavelength was found to be 265nm. The calibration curve of *Acalypha indica* extract was constructed by dissolving the drug in pH 7.2 phosphate buffer. The calibration curve obtained is shown in **Table no.2** and **Figure no.2**.

Table no. 2: Calibration curve data of *Acalypha indica* extract.

Concentration ($\mu\text{g/ml}$)	Absorbance at 320nm
0	0
5	0.114
10	0.214
15	0.329
20	0.423
25	0.508
30	0.619

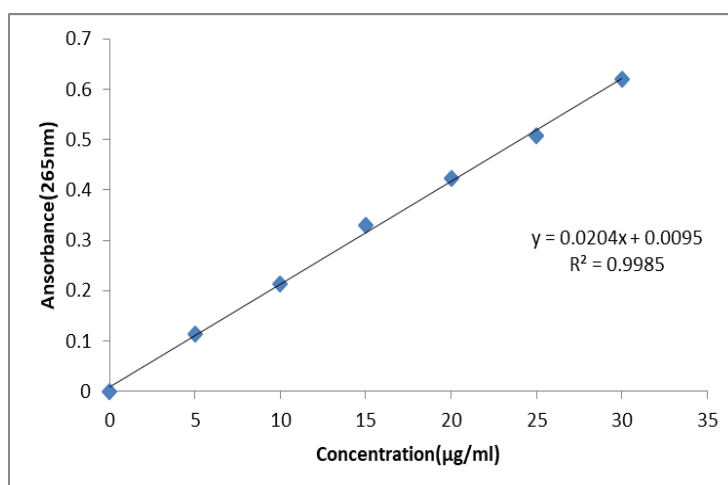


Figure no. 3: Calibration curve of *Acalypha indica* extract.

Evaluation of *Acalypha indica* complex

Optical microscopy

Optical microscopy was carried out by viewing the formulations under optical microscope. It was observed that the vesicles formed and were found to be of uniform size and shape.



Figure no. 4: Microscopic view of *Acalypha indica* phytosomes.

FT-IR Studies

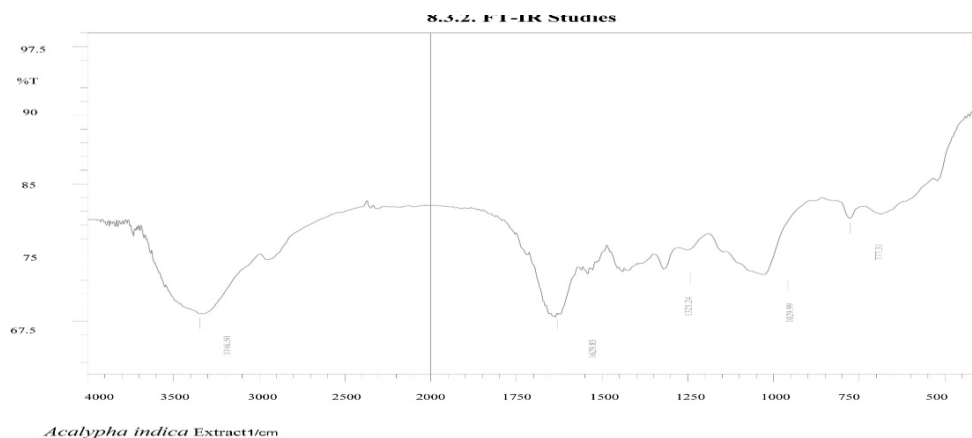


Figure no. 5: FT-IR Studies of *Acalypha indica* extract.

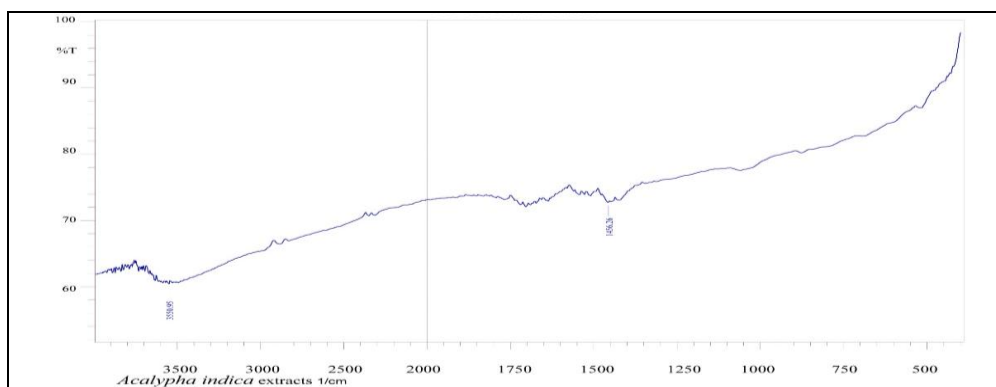


Figure no. 6: FT-IR studies of *Acalypha indica* gel.

The FTIR spectrum of *Acalypha indica* extract and the formulation were shown in **Figure 5 & 6**. From this, the complex formation was indicated by the formation of strong hydrogen bonding between hydroxyl group of phospholipids and extract phytoconstituents in *Acalypha indica* herbal gel form, and it showed that the excipients are more compatible.

Percentage practical yield

Table no. 3: Results of percentage practical yield.

Formulation	Percentage Practical Yield
F1	90.28
F2	88.15
F3	86.51
F4	96.65
F5	82.47
F6	93.72
F7	80.69

Percentage Practical yield of different formulations were shown in **Table no 3**. F4 have higher Percentage Practical yield of 96.65%.

Entrapment efficiency

Table no. 4: Results of entrapment efficiency.

Formulation	Percentage entrapment efficiency
F1	86.10
F2	83.94
F3	78.38
F4	92.25
F5	70.91
F6	89.71
F7	66.15

The entrapment efficiency was calculated from the absorbance obtained from the supernatant solution. The formulation F4 showed highest release entrapment efficiency of 92.25%.

Drug content

Table no. 5: Results of drug content.

Formulation	Drug Content (% W/W)
F1	87.03
F2	86.41
F3	85.15
F4	93.65
F5	81.47
F6	89.86

F7	79.36
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The drug content of *Acalypha indica* extract in the complexes was found to be in the range of 79.36% - 93.65%. The formulation F4 showed the maximum drug content of 93.65%. The results were shown in **Table no 5**.

***In-vitro* drug diffusion study of herbal gel**

Table no. 6: Results of *in-vitro* drug diffusion study.

Time in hours	F1	F2	F3	F4	F5	F6	F7
1	14.32	11.21	13.21	18.63	11.26	12.43	12.89
2	27.12	23.43	22.34	32.63	25.89	26.71	26.22
3	44.23	48.71	45.21	54.60	51.23	50.21	50.33
4	67.23	68.43	70.33	72.21	71.34	69.33	70.87
5	78.22	80.21	81.34	87.12	83.24	79.23	84.23
6	87.12	88.45	89.25	92.23	90.12	88.56	90.12
7	91.23	90.46	93.34	97.13	92.13	96.54	92.67
8	94.33	92.79	94.34	99.8	93.33	97.03	95.46

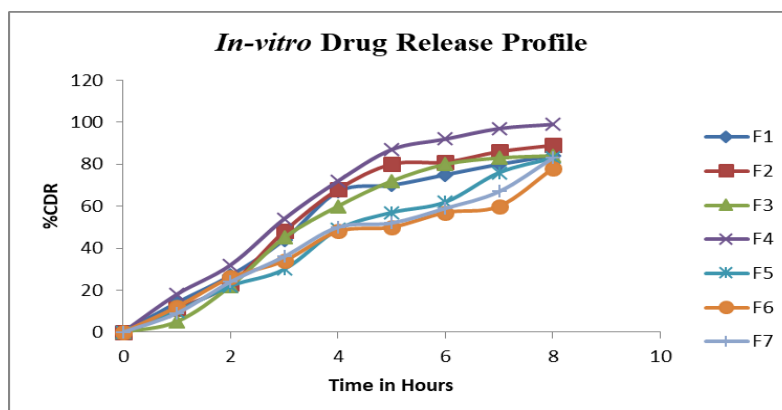


Figure no. 7: *In-vitro* drug diffusion profile.

Anti -Bacterial activity

Table no. 7: Results of Anti-bacterial activity.

Sl. no.	Test parameter	Method	Results (Zone of Inhibition in mm)		
			<i>Acalypha indica</i> extract		Gentamycin (120mcg)
			50 μ l	100 μ l	
1	<i>S.aureus</i>	Lab method	13	14	18 mm

The anti-microbial activities of *Acalypha indica* formulation against the microorganism were examined in the present study, and the potency was assessed by the presence or absence of inhibition zone and zone diameter is compared with gentamycin. Results are given in **Table no.7**. The results showed that the formulation was effective to kill bacteria.

Scanning Electron Microscopy (SEM) Analysis

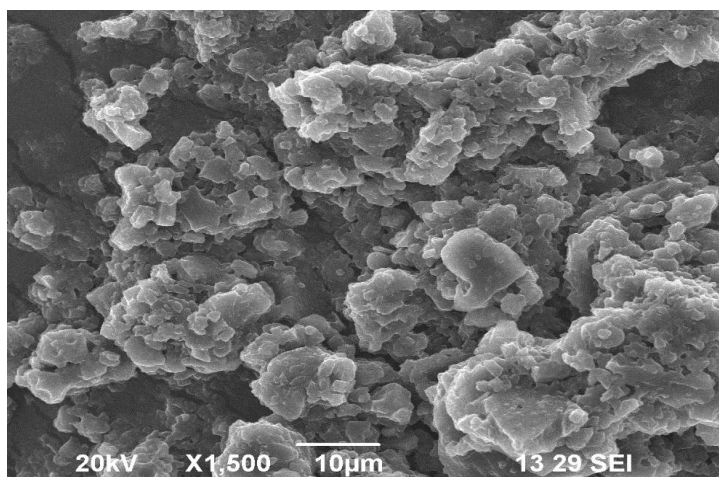


Figure no. 8: SEM image of aqueous extract of *Acalypha indica* herbal gel (F4).

Scanning electron microscopy showed that the vesicles are spherical in shape and smooth in nature.

Evaluation of *Acalypha indica* gel

Homogeneity

Table no. 8: Results of homogeneity of optimized gel formulation.

Formulation	Homogeneity
Optimized gel	Good

From the visual inspection of the prepared gel formulation (F4) showed good appearance and homogeneity.

Measurement of pH

Table no. 9: Result of pH of optimized gel formulation.

Formulation	Ph
Optimized gel	5.8

The pH of optimized gel formulation was 5.8

Drug content

Table no. 10: Result of drug content of different gel formulations.

Formulation	Drug Content (%)
Optimized gel	93.65

The drug content of the optimized gel was estimated spectrophotometrically at 265nm. The drug content in which the best formulation contained 93.65% showed in the **Table no.10**.

Rheological study

Table no. 11: Result of viscosity of optimized gel formulation.

Formulation	Viscosity (Centipoise)
Optimized gel	9673

The viscosity of the optimized gel formulation was found by the Brookfield's viscometer. The gel was rotated at 50 rpm for 10min. The corresponding reading was noted. The viscosity of the formulations increases as concentration of polymer increases. The viscosity of the optimized gel was found to be 9673 centipoise.

Spreadability

Table no. 12: Results of spreadability of optimized gel formulation.

Formulation	Spreadability (cm)
Optimized gel	5.5

Spreadability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. The spreadability of the fabricated gel formulation was carried out and it was found to be 5.5cm which denoted that it has good spreadability.

Extrudability

Table no. 13: Results of extrudability of optimized gel formulation.

Formulation	Extrudability (gm/cm ²)
Optimized gel	9.6

The optimized gel formulation was shown optimum extrudability. Because, the extrudability was decreased with in the concentration of gelling agent.

In-vitro drug release study

The *in-vitro* release studies of the optimized formulation were carried out using Franz diffusion cell. The egg membrane was mounted between the donor and receptor compartment. The receptor compartment contains 15ml of pH 7.2 phosphate buffer and the temperature was maintained at 37°C and was constantly stirred. The samples were withdrawn at specific time intervals and they were analyzed at 265nm using UV spectrophotometer.

Stability studies

Table no. 14: Stability studies for optimized gel formulation.

Sl. No.	Parameters	Initial	30 th Day	45 th Day
1	Homogeneity	Good	Good	Good
2	Drug Content (%)	93.71	93.67	93.64
3	pH	5.8	5.8	5.8
4	Spreadability	5.5	5.5	5.4
5	Extrudability (gm/cm ²)	9.1	9.1	9.1
6	Viscosity (cp)	9673	9658	9630
7	% Cumulative drug Release	98.85	98.80	97.71

For the optimized gel formulation i.e stability study was carried out as per ICH guideline. There were no marked changes in the optimized gel formulation. The results of homogeneity, drug content, pH, spreadability, extrudability, viscosity, % cumulative drug release of the formulation was related to that of initial reference. The results were shown in **Table no.14** and it showed that the formulated gel was stable during the stability studies.

CONCLUSION

Acalypha Indica has many medicinal activities and the extract of *Acalypha Indica* was converted into gel by using carbopol as base. The results of homogeneity, drug content, pH, spreadability, extrudability, viscosity, % cumulative drug release were good. The fabricated *Acalypha Indica* gel showed anti bacterial activity against *S.aureus*. It can be concluded that, the fabricated gel may be used in the management of skin infections.

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