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Formulation and Evaluation of Herbal Anti-Inflammatory Phytosome Gel Containing Methanolic Extract of Crotalaria Biflora

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ABSTRACT: Crotalaria is a genus of flowering plants in the family Fabaceae, commonly known as rattlepods. The genus includes over 700 species of herbaceous plants and shrubs. Several species of Crotalaria are cultivated and consumed across the world by the rural population for a variety of purposes that include medicine, food, green manure, fodder etc.

The present study highlights the formulation & evaluation of a herbal topical anti – inflammatory phytosome gel containing methanolic extract of Crotalaria biflora. Extracts of whole plant was prepared by maceration with methanol as solvent. Crotalariabifloraphytosome was prepared by rotary evaporation technique using plant extract and phosphatidylcholine. The phytosomal formulation was subjected to various studies like organoleptic evaluation, entrapment efficiency & FTIR studies was conducted.

Phytosome was formulated as a gel and evaluated for various physico chemical parameters such as homogeneity, pH, viscosity, spreadability, extrudability. From the prepared formulation the best formulation was F1 based on various evaluation parameters. Limit test for heavy metals, identification test and microbial limit was carried out. Diffusion study and in vitro anti-inflammatory activity was also carried out.

KEYWORDS: Crotalaria biflora, Phytosomal gel, Anti- inflammatory activity

I. INTRODUCTION

Herbal medicine is one of the oldest and most widely used health-care systems. According to the World Health Organization (WHO), 80 percent of the world's populations currently use herbal medicine for primary health care.

In the past decades considerable attention has been focused on the development of novel drug delivery systems for herbal drugs.

The genus Crotalaria belongs to the family Fabaceae, commonly known as rattlebox. The common name rattlepod or rattlebox is derived from the fact that the seeds become loose in the pod as they mature, and rattle when the pod is shaken.

It is commonly used as hepatoprotective, deworming in malaria treatment, management of fever, pesticide in agriculture, antimicrobial, antioxidant, anti-inflammatory etc. In India,Crotalaria plants are commonly found in the fields and forest lands throughout the year and sometimes cultivated.[5]

Phytosomes are structures which contain the active ingredients of herb surrounded and bound by phospholipids. These are the newly introduced technology developed to incorporate standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, called as phytosomes. It results from reaction of stoichiometric amount of phospholipid mostly phosphatidylcholine with a standardized herbal extract in an aprotic solvent. It mainly contains the bioactive phytoconstituents of herb surrounds and bound by a lipid. These are prepared through the attachment of individual ingredients of herbal extracts to phosphatidylcholine, resulting in a formulation having higher solubility and hence better absorption leading to promoted pharmacokinetic and pharmacodynamic properties compared to the conventional herbal extracts. [10,11]

Benefits of Phytosomes

1. Potential enhancement of bioavailability.

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2. Herbal phytosome act as a cell whereby the valuable components of the herbal extracts are protected from destruction by digestive secretions and gut bacteria in the body.
3. Pharmacologically assured delivery to the different biological tissues.
4. Less dose is only required due to absorption of chief constituent.
5. Drug loading efficiency is very high and more over predetermined because drug itself in conjugation with lipids forms vesicles.
6. Significantly enhanced ability of phyosome to cross cell membranes and enter cells.
7. Drug entrapment is effective.
8. Phytosomes shows better stability profile due to formation of chemical bond between phosphatidylcholine molecules and phytoconstituents.
9. Phosphatidylcholine used in the phytosome process acts as a carrier and also nourishes the skin, because it is essential part of cell membrane.
10. Significantly gives greater clinical benefit than other forms of drug delivery systems12, 13.

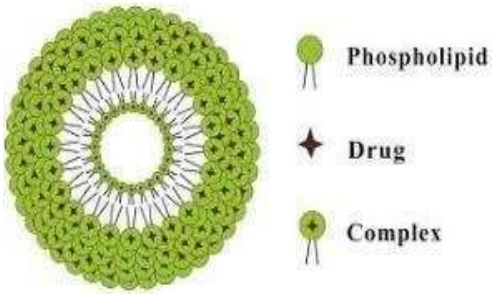

Fig No: 1 Phytosome complex

Herbal gel

A gel is a solid or semisolid system of atleast two constituents, consisting of a condensed mass enclosing and interpenetrated by a liquid.

Gel can be formulated mainly by 3 methods:

- Fusion method
- Cold method
- Dispersion method

Gel formulations have many advantages such as;

- Avoidance of first pass metabolism
- Convenient and easy to apply
- Improve patient compliance
- Provide suitability for self-medication[15,16]

Anti-inflammatory activity

Inflammation is a localized physical condition in which part of the body becomes reddened, swollen, hot, and often painful, especially as a reaction to injury or infection.[14] Anti-inflammatory drug inhibits cyclooxygenase (COX), the enzyme that catalyses the synthesis of cyclic endoperoxides, from the arachidonic acid to form PGs. The two COX isoenzymes are COX-1and COX-2. The function of COX-1 is to produce PGs that are involved in normal cellular activity, (protection of gastric mucosa, maintenance of kidney function). While; COX-2 is responsible for the production of PGs at the inflammation sites.[17]

In the present study, the whole plant of Crotalaria biflora was collected from the Mekkarai, the village located near the foothills of Western Ghats in Tirunelveli District, Tamil Nadu in Southern India. The collected plant material was subjected to extraction. Our present study was aimed to formulate and evaluate herbal antiinflammatoryphytosomal gel containing the methanolic extract of whole plant of Crotalaria biflora.

Plant profile

- Botanical source: Crotalaria biflora
- Family: Fabaceae
- Synonym: Rattlebox
- Geographical source: Mekkarai, Tirunelveli (dis), Tamil Nadu, South India.

1.7.1 Taxonomical representation

- Kingdom: Plantae
- Phylum: Tracheophyta
- Class: Equisetopsida
- Order: Fabales
- Family: Fabaceae
- Genus: Crotalaria
- Species: Crotalaria biflora L.

Characteristics

Crotalaria biflora has alternate leaves. The leaves are petiolate and entire.

Plant produces pea – shaped flowers that are arranged in racemes. At root produces legumes.[19,20]

AIM AND OBJECTIVE

AIM:

To formulate anti-inflammatory phytosomal gel of methanolic extract of whole plant of Crotalaria biflora.

OBJECTIVE:

- Collection, authentication and drying of Crotalaria biflora
- Preparation of total methanolic extract of whole plant of Crotalaria biflora.
- Formulation of phytosome.

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- Pre-formulation studies.
- Formulation of anti- inflammatory gel.
- Evaluation of anti- inflammatory gel.
- Comparative study on prepared formulation and marketed formulation.

II. EXPERIMENTATION

2.1 Plant Collection and Authentification

The whole plant of Crotalaria biflorawas collected from the Mekkarai, a village close to the foothills of the Western Ghats in Tirunelveli district of Tamil Nadu, southern India. The collected plant material was identified and authentified. The plant materials were dried under shade for few days, powdered with mechanical grinder and stored in an airtight container.

2.2 Extraction

Powdered plant material was subjected to maceration with methanol as solvent. 50 g of drug was placed inside a container, the menstruum is poured on its top until it completely covers the drug material. The container is then closed and kept for five days. The content is then stirred periodically; after maceration, the extract was filtrated and the solvent was removed by evaporation to dryness on a water bath and it was then stored in a desiccator.[31]

Fig no: 4 Maceration of whole plant of Crotalaria biflora

2.3 Formulation of phytosomes

To prepare the phytosomes of Crotalaria biflora, 100mg drug extract, 100 mg phosphatidylcholine and 50ml ethanol were taken in the flask of vacuum rotary evaporator. The mixture was shaken at a temperature not exceeding 60˚C for 2 hours. The phytosomes were precipitated and ethanol was evaporated under vacuum to remove the traces of solvent. The dried residues were gathered and placed in a desiccator.[32]

Fig no: 5 Preparation of phytosome

2.4 Pre- formulation studies of phytosome:

2.4.1 Determination of entrapment efficiency (EE %):100 mg of Crotalaria bifloraphytosomal complex were centrifuged at 2000 rpm for 30 min using a centrifuge to separate phytosomes from unentrapped drug. Concentration of the free drug as the supernatant was determined by measuring absorbance at 279 nm using UV-Visible spectrophotometer. The percentage drug entrapment was calculated by using the formula, [33]

Entrapment efficiency (%)	=	Total amount of drug - amount of free drug / Total amount of drug	x 100

2.4.2 Organoleptic characteristics:

The phytosome was characterized for the physical characterization like appearance, colour and odour. [34]

2.4.3 FTIR spectroscopy

The compatibility studies were carried out at room temperature using FTIR spectroscopy to determine the interaction of drug with excipients used in the formulations and phytosome. The IR spectrum of drug alone was taken. Physical mixtures of the excipients in the ratio 1:1 was prepared and the samples were analyzed in IR spectra analyzer.

2.5 Formulation of phytosomal gel

- **Preparation of gel**: Carbopol 934 was dissolved slowly with 60 ml of distilled water by stirring for 1hr to avoid agglomeration. Then methyl paraben and triethanolamine were dissolved in 10 ml of distilled water separately and stirred for 10 min. Mixed 4.83 ml of propylene glycol in 12 ml of distilled water by

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stirring continuously for 10 min. Methyl paraben and triethanolamine solution were added to the carbopol solution and the pH was then adjusted to 7.4. Then propylene glycol solution was added with continuous stirring for 10 min, until a clear consistent gel base was obtained.

- **Incorporation of phytosomal complex into the gel:** The solution of phytosomal complex was prepared by using 0.1 ml of ethanol in a beaker and it was added to the Carbopol base. Prepared gels were stored in suitable containers at room temperature for further studies.[35, 36]

Ingredients	F1 (g)	F2(g)
Carbopol 934	1.5	1.5
Triethanolamine	1.5	1.5
Propylene glycol	5	5
Methyl paraben	0.005	0.005
Phytosome	1	0.5
Distilled Water	q.s	q.s

Table no: 1 Composition of phytosomal gel

2.6 Evaluation of phytosomal gel

2.6.1 Homogeneity

All developed phytosomal gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.[37]

2.6.2 Measurement of pH

The pH of the phytosome gels were measured with the help of digital pH meter. 0.5 g of phytosome gel was dissolved in 25 ml of distilled water and was kept for two hrs. Then the pH of each formulation was determined.[38]

2.6.3 Rheological study

The viscosities of prepared gels were carried out by using Brookfield viscometer (spindle type T-95). 50 g of gel was filled in a 100 ml beaker and viscosity was measured at 2.5, 4, 5 and 10 rpm. The readings of each formulation were taken.[39]

2.6.4 Spreadability

Spreadability was determined by the multimer apparatus which consists of a wooden block, which was provided by a pulley at one end. Spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel 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. 1kg weight 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 by 60 gms weight. 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.[40]

Spreadability was calculated using the following formula:

$S = M \times L / T$

Where,S = Spreadability, M = Weight tied to the upper plate, L = Length through which the gel spreads (7.5cm), T = Time (in sec) taken for separating

2.6.5 Extrudability

In the present study, extrudability was determined by measuring the weight required to extrude at least 0.5cm gel from lacquered aluminum collapsible tube in 10 secs. The extrudability was then calculated by using the following equation:[41]

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

2.7 Pharmacognostic evaluation

2.7.1 Determination of extractive values

Alcohol soluble extractive value

5 grams of formulation with 100 ml ethanol were taken in a stoppered flask, for 24 hours with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours. 25 ml of the filtrate was evaporated to dryness and weighed.

Ether soluble extractive value

5g of formulation was extracted with ethyl ether in a soxhlet extractor for 20 hrs.

Ether extract was transferred in a petridish and allowed to evaporate. It was dried at 105℃ and weighed.[42]

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2.7.2 Test for identification

1. Test for Alkaloids

a) Mayer's Test

2ml of the filtrate was shaken with an equal quantity of Mayer's reagent and observed for the formation of creamy precipitate.

b) Wagner's Test

2ml of the filtrate was mixed with an equal quantity of Wagner's reagent and observed for the formation of a reddish-brown precipitate.

c) Hager's Test

1ml of Hager's reagent was added to the 2ml of filtrate and observed for the formation of yellow precipitate.

d) Dragendorff's Test

2ml of the filtrate was treated with an equal quantity of Dragendorff's reagent and observed for the formation of the orange-red precipitate. **2. Test for Phenolic compounds and Tannins**

a) Ferric chloride Test

A small quantity of formulation was treated with a 5% dilute ferric chloride solution and observed for the formation of blue colour.

b) Gelatin Test

2-3 drops of dried extract were mixed with water and filtered. To the filtrate 2% gelatin containing 10% sodium chloride solution was added and observed for the formation of milky white precipitate.

c) Lead acetate Test

2-3 drops of dried extract in water was treated with a 10% lead acetate solution and observed for the formation of bulky white precipitate.

3. Test for Terpenoids

a) Salkowski's Test

A small quantity of formulation was mixed with chloroform. To this mixture, equal quantity of concentrated sulphuric acid was added. Formation of red colour in the chloroform layer and greenish-yellow fluorescence in the acid layer.

4. Test for Saponins

a) Foam Test (Froth test)

A small quantity of formulation was mixed with 20 ml of distilled water and shaken for 15minutes and observed for the formation of foam.

5. Test for Sterols

A small quantity of the formulation was mixed with alcohol and potassium hydroxide solution for saponification. The saponified mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated and the residue obtained was used for the detection of sterols.

a) Liebermann-Burchard Test

A small quantity of the residue, 2ml of chloroform and acetic anhydride was added and concentrated sulphuric acid was added to it at the sides of the test tube. Formation of green colour in the upper portion which changes to blue or violet colour.

b) Salkowski's Test

A small quantity of residue was treated with 2ml of chloroform and concentrated sulphuric acid was added to this mixture and observed for the formation of red colour in the lower layer.

6. Test for flavanones and flavanoids

a)Aqueous sodium hydroxide test

To the formulation add aqueous sodium hydroxide solution and observed for slight yellow colour.

b) Filter paper was wetted with gel and the filter paper was exposed to ammonia vapours, noted the yellow colour on the filter paper.[43]

2.7.3 Limit test for heavy metals

Test sample	Standard compound
Specified quantity of samplewas taken (2 ml), dissolved in 20 ml of water.	Specified quantity of sample was taken (2 ml), dissolved in 20 ml of water.
Add 5 ml of sodium hydroxide.	Add 5 ml of sodium hydroxide.
Add 5 drops of sodium sulphide solution.	Add 5 drops of sodium sulphide solution.
Make up the volume upto50ml with distilled water.	Make up the volume upto50mlwith distilled water.

Keep aside for 5 mins. compare the intensity of colour produced by the test as that of the standard.[44]

2.8 Microbial limit

The formulation was inoculated in Mueller Hinton agar. It has to be incubated at 3537^{0}C for 24 hours. If there is no growth of the microorganism, then the formulation passes the test. The presence of microorganism will be detected with the presence of colonies surrounded by a clear zone.[45]

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2.9 In vitro anti-inflammatory study

2.9.1 Drug Diffusion Study

The in vitro diffusion study was done by using Franz diffusion cell. The egg membrane was mounted between the donor and receptor compartment. The receptor compartment was filled with 15 ml of pH 7.4 phosphate buffer maintained at 37˚C and was constantly stirred by using a magnetic stirrer. 1g of phytosomal gel was placed on the egg membrane. At each sampling interval, samples were withdrawn for a period of 1 hr and were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analyzed spectrophotometrically at 279 nm.46

Solvent Used for maceration	Colour	Consistency	Weight of extract (g)	Percentage yield (% W/W)
Methanol	Brown	Gel	4.54	9.08

2.9.2 Protein denaturation method

The reaction mixture (0.5ml) consists of 0.45 ml bovine serum albumin and 0.05ml of plant extracts of 50, 100, 150, 200 mcg/ml concentrations. The pH was adjusted to 6.3 using 1N HCl. The samples were incubated at 37^0C for 20 min. and then heated at 57^0C for 3min. Diclofenac gel used as standard drug (50,100,150,200$\mu g/ml$). After cooling the samples 2.5ml phosphate buffer saline (pH 6.3) was added to each test tube. Absorbance was measured spectrophotometrically at 660nm. [47,48]

The percentage inhibition of protein denaturation was calculated as follows;

Percentage inhibition = $[(A0 - A1)/A0] \times 100$

Where; A0 = Absorbance of the control
A1 = Absorbance of the sample

2.11 Stability studies

Stability of a drug in a dosage form at different environmental conditions is important, because it determines the expiry date of that formulation. Hence, the stability of the prepared formulation was studied. Stability studies were conducted according by storing the gel formulation at 40^oC ± 20^0C, 70% RH ± 5% for 30 days. The samples were withdrawn at initial, 5th, 10th, 15th& 20th day and analyzed suitably for the physical characteristics. [49, 50]

III. RESULTS AND DISCUSSION

3.1 Authentification

The whole plant of Crotalaria biflora was collected from the Mekkarai, a village close to the foothills of the Western Ghats in Tirunelveli district of Tamil Nadu, Southern India. The collected plant material was authentified by retired research officer V. Chelladurai, Central Council for Research in Ayurveda & Siddha, Govt. of India. It was then subjected to shade drying.

3.2 Extraction of whole plant Crotalaria biflora

The dried powder of Crotalaria biflora was subjected to maceration using methanol as solvent. After extraction the percentage yield was calculated.

Table no: 2 Results of % yield of the extract

3.3 Evaluation of Phytosomal complex

3.3.1 Entrapment Efficiency

The entrapment efficiency was calculated from the absorbance obtained from the supernatant solution. The entrapment efficiency of phytosome was found to be 97%

3.3.2 Organoleptic characters

Table no: 3 Results of organoleptic characters of phytosome

Sl no.	Characteristics	Observation
1	Colour	Greenish brown
2	Odour	Pleasant

3.3.3 FTIR spectroscopy

The FT-IR spectrum of the crude drug extract, phytosome and formulation (F1) was shown in fig no(18,19,20) and the peak values obtained in table no.(8,9,10). The peak values were found to be identical with reference.

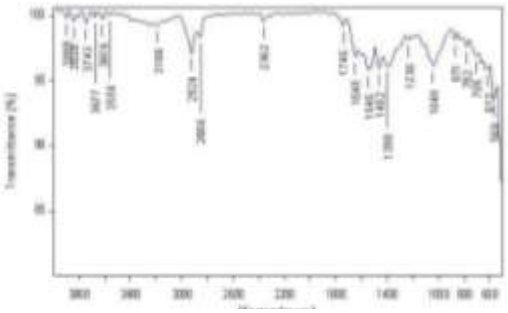

Fig no: 6 FTIR spectra of theextract

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Functional group	Characteristic peak	Observed peak
Aromatic (C=C)	1500-1450	1462
Amide	1870-1540	1648
Alkane	3000-2850	2856
Alcohol	3600-3500	3554
Aldehyde	2800-2860	2856

Table no: 4 FTIR spectra of the extract

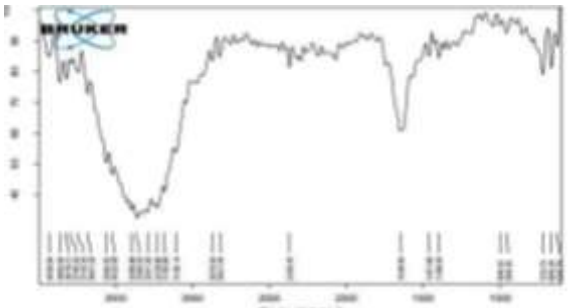

Fig no: 7 FTIR spectrum of methanolic extract of Crotalaria biflora& excipients.

Functional group	Characteristic peak	Observed peak
Aromatic(C=C)	1500-1450	1461
Amide	1870-1540	1646.85
Alkane	3000-2850	2870
Alcohol	3600-3500	3560
Aldehyde	2800-2860	2821.45

Table no: 5 FTIR spectrum of methanolic extract of Crotalaria biflora and excipients

3.4 Evaluation of phytosomal gel complex

Sl no.	Parameters	F1	F2
1	Homogeneity	Good	Good
2	pH	7.42	7.42
3	Rheological study	3.9 Cp	3.9 Cp
4	Spreadability	4.1g.cm/sec.	3.8g.cm/sec.
5	Extrudability	9.3g.cm/sec.	10.3g.cm/sec.

Table no: 7 Results of evaluation of phytosomal gel formulations

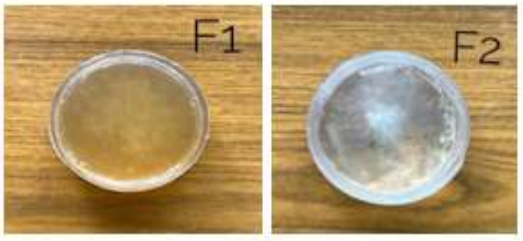

Fig no: 9 Homogeneity study

Fig no.10 pH meter

Fig no: 11 Rheological study

Fig no: 12 Spreadability study
Fig no: 13 Extrudability study (Multimer apparatus)

3.5.1 Determination of extractive values

Sl no.	Parameter	Average (%w/w)
1	Alcohol soluble extractive value	2.40
2	Ether soluble extractive value	1.22

Sl no.	Chemical test	Result
1	**Alkaloids**	
a	Mayer's test	++
b	Wagner's test	++
c	Hager's test	++
d	Dragendorff's test	++
2	Phenolic compounds	
a	Ferric chloride test	+++
b	Gelatin test	++
c	Lead acetate test	++
3	**Terpenoids**	
a	Salkowski's test	+
4	**Saponins**	
a	Foams test	+
5	**Sterols**	

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a	Liebermann-Burchard Test	+
b	Salkowski's Test	+
6	**Flavanones and flavanoids**	
a	Aq.sodium hydroxide test	++
b	Ammonia vapour test	++

3.5.3 Microbial limit

The microbial limit test for both the gels were conducted and after 24 hours, no colony forming unites were observed at a temperature of 37^0C.

Fig no: 20 Result of microbial limit.

3.5.4 Limit test for heavy metals

The intensity of color produced in the test is less than that of standard, then the given sample was passes the limit test.

3.6 In vitro drug release study

3.6.1 Diffusion study

Time (hr)	Drug release
0.5	10.36
1.5	12.63
2	24.1
4	35.17
8	59.34
24	89.35

Table no .11 Result showing in vitro drug release study.

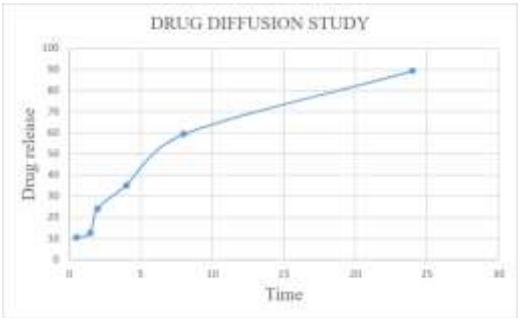

Fig no.22 Result showing diffusion study

In vitro drug release profile of the selected formulation (F1) was studied in phosphate buffer of pH 7.4 using diffusion tube. The cumulative drug release profile for 24 hrs is shown in the table no: 11. At 24^{th} hr the formulation shows 89.35% drug release.

3.6.2 Protein denaturation

Sl no.	Sample	Conc. (mcg/ml)	Absorb-ance (660nm)	% inhibi-tion (mcg/ml)
1	Control	-	0.812	-
2	Test	50 100 150 200	0.553 0.413 0.292 0.229	32.06 49.26 64.12 71.86
3	Standard (Diclofenac gel)	50 100 150 200	0.480 0.342 0.236 0.150	40.90 57.88 70.94 81.53

Table no: 12 Results showing percentage inhibition of protein denaturation

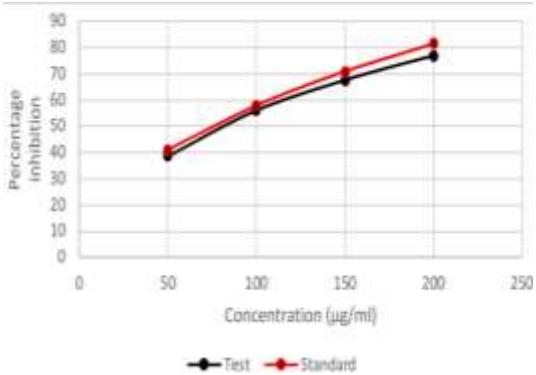

Fig no: 23 % inhibition v/s conc graph

IC_{50} value of test = 60.057µg/ml

IC_{50} value of standard = 75.585µg/ml

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3.7 Accelerated stability study

Day	Temperature	pH	Homogeneity	Spreadability	Extrudability
0	RT 40°C±2°C 70±5%	7.4 7.4	Good Good	4.1 4.1	9.3 9.3
5	RT 40°C±2°C 70±5%	7.4 7.4	Good Good	4.1 4.1	9.3 9.3
10	RT 40°C±2°C 70±5%	7.4 7.4	Good Good	4.1 4.1	9.3 9.3
15	RT 40°C±2°C 70±5%	7.4 7.4	Good Good	4.1 4.1	9.3 9.3
20	RT 40°C±2°C 70±5%	7.4 7.4	Good Good	4.1 4.1	9.3 9.3

Table no: 13 Results of accelerated stability study of phytosomal gel

IV. SUMMARY & CONCLUSION

Crotalaria biflorais an herb belonging to the family fabaceae. It was collected from the Mekkarai, a village close to the foothills of the Western Ghats in Tirunelveli district of Tamil Nadu, Southern India. Scientific investigations have reported its antioxidant, antimicrobial, antiinflammatory, hepato-protective activities.

Extraction of dried powder of the whole plant of Crotalaria biflora was carried out by maceration by using methanol as solvent.Crotalaria bifloraphytosome was prepared by rotary evaporation technique using plant extract and phosphatidylcholine. The phytosomal formulations were subjected to various studies like organoleptic evaluation, entrapment efficiency and FTIR.

The physicochemical parameters such as homogeneity, pH,viscosity, spreadability and extrudability were carried out. Pharmacognostic evaluation such as extractive values, limit test for heavy metals, identification tests, and microbial limit were also carried out. Also, the presence of alkaloids, phenolic compounds, terpenoids, saponins, sterols, flavanones and flavanoids was obtained from the identification test. The microbial limit was conducted, no colony forming units were observed.

From the prepared formulations, the best formulation was found to be F1 based on the various parameters such as spreadability and extrudability. In vitro drug release study such as diffusion and protein denaturation were also carried out. Diffusion study of F1formulation was studied in phosphate buffer (pH 7.4) and cumulative drug release profile for 24hrs.was found. On observation and analysis of the obtained result, the formulation was found to possess antiinflammatory activity.

As per our studies and obtained results, the methanolic extract of Crotalaria biflora can be successfully formulated into herbal topical anti-inflammatory phytosomal gel and possess significant topical anti-inflammatory properties.

REFERENCE

[1]. Manisha Yadav, Vidhi J, Bhatia, Gaurav Doshi. Novel techniques in herbal drug delivery system, International Journal of Pharmaceutical Science.2014; 1(6): 70-83.

[2]. Jamadar M.J, Shaikh R.H. Preparation and evaluation of herbal gel formulation. Journal of Pharmaceutical Research and Education. 2017; 1(2):201-204.

[3]. Anju Dhiman, Arun Nanda, Sayeed Ahmed. Novel herbal drug delivery system, International Conference on Environmental Chemistry and Biology. 2012;49(34):171-172

[4]. Bhatt K.C, Pandey A, Dhariwal O.P, Panwar N.S, Bhandari.“Tum-thang” (Crotalaria tetragonaRoxb). A little known wild edible species in the north-eastern hill region of India. Genetic Resources and Crop Evolution. 2009; 56(5):729-733.

[5]. Devendra B.N, Srinivas N, Solmon K.S. A comparative pharmacological and phytochemical analysis of in vivo & in vitro propagated Crotalaria species. Asian Pacific Journal of Tropical Medicine. 2012; 5(1):37-41.

[6]. Anoob Kumar KI, Swamivel Manickam M, Sreejith M, Sebastin V. Preliminary phytochemical evaluation, isolation and spectroscopic characterization of constituents in the dried extracts of the whole plant Crotalaria biflora (L),

International Journal of Pharmaceutical Science. 2020; 10(5):124-133.

[7]. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chinese Medicine. 2018; 13(1):1-26.

[8]. Azwanida N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal Aromatic Plants. 2015; 4(196):2167-2188.

[9]. Handa SS. An overview of extraction techniques for medicinal and aromatic plants. Extraction Technologies for Medicinal and Aromatic Plants. 2008; 1 (9):21-40.

[10]. Joseph A. Kareparamban, Pravin H, Aruna P Jadhav, Vilasrao J. Phytosome a novel revolution in herbal drugs, International Journal of Pharmaceutical Science Chemistry. 2012;2(2):299-300.

[11]. G. Monica and V.Vasu Naik, Herbosomes. A potential carriers for the bioavailability enchancement of herbal extracts, WJPPS. 2014; 4(1):1052-1065.

[12]. Patel Amit, Tanwar Y.S, Suman Rakesh, Patel poojan. Phytosome- Phytolipid drug delivery system for improving bioavailability of herbal drug, International Journal of Pharmaceutical Science 2013;3(2):51-52

[13]. G.S Ravi, V Chandur, A.R Shabaraya, K Sanjay. Phytosomes - An advanced herbal drug delivery system, International Journal of Pharmaceutical Science. 2015;4(3):416-417

[14]. Anne Waugh, Allison Grant.. Anatomy and physiology of skin, anatomy and physiology in health and illness. Ross and Wilson 1987;3 (1):358-359

[15]. Rajashekar K, Sundari P, Srinivas D. Development of a topical phytosomal gel of wood fordiafruticosa. World Journal Pharmaceutical Science. 2015;4(11):19-32.

[16]. Ingle S, Tegeli V, Chandakavate B, Matole V, Kirdak O, Gophane G, Nangare S, Adlinge S, Ramanshetti S. A novel topical drug delivery system. Research Journal of Pharmaceutical Dosage forms and Technology. 2021; 13 (1):25-30.

[17]. Ricciotti E, Gerald G. Prostaglandins and inflammation, Arteriosclerosis, thrombosis, and vascular biology. 2011;31 (5):986-1000.

[18]. Stankov SV. Definition of inflammation causes of inflammation and possible antiinflammatory strategies. The Open Inflammation Journal. 2012; 5(1):1-9.

[19]. Rajakaruna N, Harris C.S, Towers G.H. Antimicrobial activity of plants collected from serpentine outcrops in Sri Lanka. Pharmaceutical Biology. 2002; 40 (3):235-44.

[20]. Kumar K.A, Manickam M.S, Sreejith M, Sebastin V. In vitro antioxidant and anthelmintic activity of the extracts of whole plant Crotalaria biflora (L). Annals of the Romanian Society for Cell Biology. 2021; 7(6):150-161.

[21]. Kumar KA, Manickam MS, Sreejith M, Sebastin V. In vitro and In vivo Antiinflammatory evaluation of the whole plant extracts of Crotalaria biflora (L). Pharmacognosy Journal. 2021; 13(3):3-4.

[22]. Bailey Y.A, Williams L.A, Green C.E, Rodney S, Smith A.M. In vitro evaluation of the anti-inflammatory potential of selected jamaican plant extracts using the bovine serum albumin protein denaturation assay. International Journal of Pharmaceutical Sciences Review and Research. 2017; 47(1):145-53.

[23]. Ittadwar P.A, Puranik P.K. Novel umbelliferonephytosomesm - development and optimization using experimental design approach and evaluation of photo-protective and antioxidant activity. International Journal of Pharmaceutical Science. 2017; 9(3):218-28.

[24]. Udapurkar P, Bhusnure O, Kamble S, Biyani K. Phyto-phospholipid complex vesicles for phytoconstituents and herbal extracts: A promising drug delivery system. International Journal of Pharmaceutical Science.2016; 4(5):14-20.

[25]. Das M.K, Kalita B. Design and evaluation of phyto-phospholipid complexes (phytosomes) of rutin for transdermal application. International Journal of Pharmaceutical Science. 2014; 4(10):51-75.

[26]. Negi A, Sharma N, Singh M. Formulation and evaluation of an herbal antiinflammatory gel containing eupatorium leaves extract. Journal of Pharmacognosy and Phytochemistry. 2012; 1(4):112-117.

[27]. Gibi M. Development and characterization of MorindaCitrifolia,phytosomal gel for topical application 2012;1(1):45-56
[28]. Rahman H.U, Mahmood R, Haris M, Rahman N. Phytochemical profiling of successive extracts of fruit and stem bark of solanum pubescens. International Journal of Pharmaceutical Science. 2014;6(2):147-153.
[29]. Serafini M.R, Santos R.C, Dos Santos J.P, Alves I.A, Gelain D.P. Morindacitrifolia Linn leaf extract possesses antioxidant activities leukocyte migration. Journal of Medicinal Food. 2011; 14(10):1159-1166.
[30]. Rouamba A, Ouédraogo V, Karama I. Ethno-medicinal use of Crotalaria retusaL.(Fabaceae), a pyrrolizidine alkaloid toxic plant. International Journal Biochemistry. 2018; 23(2):1-6.
[31]. Abubakar A.R, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmaceutical Sciences. 2020; 3(4): 24-36
[32]. Keerthi B, Pingali P.S, Srinivas P. Formulation and evaluation of capsules of ashwagandha phytosomes. International Journal of Pharmaceutical Science. 2014; 29(2):138-142.
[33]. K. Rajashekar, P. J. PrasunaSundari, Dr. Prathima Srinivas. Development of a topical phytosomal gel of WoodfordiaFruticosa, JPPS. 2015;4(11):919-931
[34]. Kalaspuria K, Malviya SJ, Patyal. Preparation & evaluation of azithromycin – phospholipid based phytosome for oral drug delivery. Journal of Pharmaceutical Sciences.2009;12(3):36-48
[35]. Sandra AuliaMardikasari, Formulation and characterization of antioxidant phytosome gel of kersen Leaves 2020:1(1);34-46
[36]. Rudra Pratap Singh and Ramakant Narke. Preparation and evaluation of phytosome of lawsone, IJPSR. 2015;6(12):5217-5225.
[37]. PrasunaSundariPingali, Prathima Srinivas, B. Madhava Reddy. Miconazole loaded novel phytosomal topical gels, WJPPS. 2015;4(10):2305-2320
[38]. K. Rajashekar, P. J. PrasunaSundari, Dr. Prathima Srinivas. Development of a topical phytosomal gel of WoodfordiaFruticosa, WJPPS. 2015;4(11):919-931.
[39]. Rudra Pratap Singh and Ramakant Narke. Preparation and evaluation of phytosome of lawsone, IJPSR. 2015;6(12):5217-5225.
[40]. Jain S, Sharma C, Khatri P, Jain A, Vaidya AN. Pharmacognostic and phytochemical investigations of the leaves of Zizyphusxylopyrus. International Journal of Pharmaceutical Science. 2011;3(2):122-5.
[41]. Anoob Kumar KI, Swamivel Manickam M, Sreejith M, Sebastin V. Preliminary phytochemical evaluation, isolation and spectroscopic characterization of constituents in the dried extracts of the whole plant Crotalaria biflora (L). Int J Life Sci Pharma Res. 2020;10(5):124-33.
[42]. Ahmed H, Ehtesham M, Rasheed N, Mohammad AS. Pharmaceutical importance and significance of limit tests. Asian Journal of Pharmaceutical Research. 2017; 7(1):30-4.
[43]. Aryal S. Mueller hinton agar (MHA)–composition, principle, uses and preparation. Microbiology 2018;3(6);117-120
[44]. Krongrawa W, Limmatvapirat S, Pongnimitprasert N, Meetam P, Limmatvapirat C. Formulation and evaluation of gels containing coconut kernel extract for topical application. Asian Journal of Pharmaceutical Sciences. 2018;13(5):415-24.
[45]. PrasunaSundariPingali, Prathima Srinivas, B. Madhava Reddy: Miconazole loaded novel phytosomal topical gels, WJPPS. 2015;4(10):2305-2320.
[46]. Swarna SK, Nivedhitha MS, Gayathri R, Selvaraj J, Madhan K. Comparative evaluation of anti-inflammatory potential of ethanolic extract of leaf, bark and flower of Tecoma stans with ibuprofen- in vitro analysis. Pharmacognosy Journal. 2019;11(5):109-115
[47]. Bajaj S, Singla D, Sakhuja N. Stability testing of pharmaceutical products. Journal of Applied Pharmaceutical Science. 2012 Mar 24;2(3):129-38.
[48]. Jamadar M.J, Shaikh R.H, Preparation and evaluation of herbal gel formulation Journal of Pharmaceutical Research and Education 2017:1(2);201-204

[49]. Jyothi D, Koland M. Formulationn and evaluation of herbal anti-inflammatory gel containing Trigonella foenum, greacumseed extract. International Journal of Pharmaceutical Science.2016;8(1) ;41-44

[50]. Leelaprakash G, Das S.M. In vitro anti-inflammatory activity of methanol extract of Enicostemma axillare. International Journal ofPharmaceutical Science 2011;3(3); 189-199