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PREPARATION AND EVALUATION OF PHYTOSOME OF LAWSONE

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Keywords:

Phytosome, Lawsone, Antifungal activity, Anti-inflammatory activity

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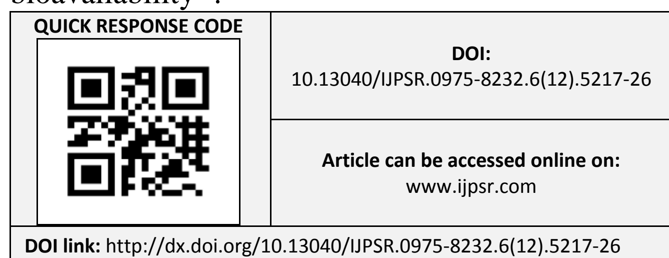
ABSTRACT: *Lawsonia inermis L.* (Lawsone) is reported to contain carbohydrates, proteins, flavonoids and phenolic compounds, alkaloids, terpenoids, coumarins, xanthones and fatty acids. The plant has also been reported to have hepatoprotective, anti-inflammatory, antibacterial, antifungal, antiviral, antioxidant and anticancer properties. Lawsone has low bioavailability because it is less soluble in water and it is rapidly eliminated from body. The aim of this study was to prepare the phytosome of lawsone and evaluate it. Different phytosome complexes of lawsone containing molar ratio of 1:1, 1:2, 2:1 and 2:2 of lawsone and soya lecithin were prepared by the antisolvent precipitation technique. The phytosome was characterized by SEM, DSC and FTIR. Antifungal activity of phytosome of lawsone was evaluated on *Candida albicans* (NCIM 3471) fungi by using ketoconazole as standard drug. The *in-vitro* permeation study was done on rat skin. The anti-inflammatory activity was evaluated in male wistar rats. SEM and DSC data showed that phytosome complex of lawsone has irregular size vesicles consisting of soya lecithin and lawsone was found to be intercalated in the lipid layer. Antifungal activity of phytosome complex (1:1) showed the biggest zone of inhibition as compared to phytosome complex (1:2), plant drug and standard drug ketoconazole after 3 days. *Ex-vivo* permeation study of phytosome gel of lawsone through excised rat skin showed 92.91% of cumulative drug permeation up to 6 h. The anti-inflammatory activity of gel of phytosome of lawsone showed significant anti-inflammatory activity as compared to plant drug gel at 4 h ($P < 0.001$).

INTRODUCTION: Preparation of plants or their parts have been widely used in medicine since ancient times and till today use of phytomedicines is widespread. Most of the biologically active constituents of plants are polar or water-soluble. However, water-soluble phytoconstituents like flavonoids, tannins, glycosidal aglycones etc. are poorly absorbed either due to their large molecular size, which cannot be absorbed by passive diffusion or due to their poor lipid solubility, thus severely limiting their ability to transport across lipid-rich biological membranes, resulting in their poor bioavailability [1].



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Phytosome is a newly introduced patented technology developed to incorporate the standardized plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, which improves their absorption and bioavailability [2]. Recent studies demonstrated the significance of this technology for standardized extracts of *Ocimum sanctum* [3], *Ginkgo biloba* [4], grape seed [5] etc. Phytosomes of *Boswellic acid* [6], silymarin [5], curcumin [6], naringenin [7] and polyphenols improved the clinical efficacy without compromising the safety for various therapeutic activities.

Lawsonia inermis L. (Lawsone) is a much branched glabrous herb or small tree, cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine. The plant is reported to contain carbohydrates, proteins, flavonoids, tannins and phenolic compounds,

alkaloids, terpenoids, quinones, coumarins, xanthones and fatty acids [8]. The plant has been reported to have analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic and anticancer properties [9].

The phospholipids soya lecithin used as a tool for heart health, coronary artery disease and improves circulation. Lecithin supplementation also helps to prevent blood clots**,** maintain the health of the liver through which excess fats and energy-providing substances pass [10]. Lecithin has a positive effect in the repair of livers damaged by any number of conditions, including excess consumption of alcohol or other toxins [11-12].

Lawsone shows low bioavailability because it is less soluble in water and other solvents and is rapidly eliminated from the body. The key objective of the present study is to develop the phytosome of lawsone to increase the solubility and bioavailability of drug. To prepared the phytosome of lawsone by specific method and evaluate its antifungal activity, *invitro* permeation study and anti-inflammatory activity.

MATERIALS AND METHODS:

Materials:

Standardized plant drug lawsone was obtained as gift sample from Yucca Enterprises, Mumbai, India. Identity of the drug was confirmed by HPLC analysis. Soya lecithin was purchased from Labin Chemicals, Pune, India. Carbopol® 934 and PEG 400 were purchased from Research lab Fine Chemicals, Mumbai, India. All other chemicals and reagents used were of analytical grade.

Animals:

Healthy Wistar male rats weighing approximately 200-250 g were used for the study. All the experimental procedures used in this study were followed by consent of the Institutional Animal Ethics Committee constituted under the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Methods:

Preparation of preliminary trial batches for selection of method:

Phytosome complex of lawsone of 1:1 molar ratio trial batches were prepared by using different methods either antisolvent precipitation technique or rotary evaporation technique or solvent evaporation technique.

1. Antisolvent precipitation technique:

The specific amount of lawsone and soya lecithin were taken into a 100 ml round bottom flask and refluxed with 20 ml of dichloromethane at a temperature not exceeding 60°C for 2 h. The mixture is concentrated to 5-10 ml. Hexane (20 ml) was added carefully with continuous stirring to get the precipitate which was filtered and collected and stored in vacuum desiccators overnight. The dried precipitate is crushed in mortar and sieved through #100 meshes. Powdered complex was placed in amber colored glass bottle and stored at room temperature [13-14].

2. Rotary evaporation technique:

The specific amount of lawsone and soya lecithin were dissolved in 30 ml of tetrahydrofuran in a rotary round bottom flask followed by stirring for 3 hours at a temperature not exceeding 40°C. Thin film of the sample was obtained to which n-hexane was added and continuously stirred using a magnetic stirrer. The precipitate obtained was collected, placed in amber colored glass bottle and stored at room temperature [13].

3. Solvent evaporation method:

The specific amount of lawsone and soya lecithin were taken into a 100 ml round bottom flask and refluxed with 20 ml of acetone at a temperature 50 - 60°C for 2 h. The mixture is concentrated to 5-10 ml to obtain the precipitate which was filtered and collected. The dried precipitate phytosome complex was placed in amber colored glass bottle and stored at room temperature [13].

Preparation of phytosome complexes of lawsone:

The different phytosome complexes of lawsone F1, F2, F3 & F4 containing molar ratio of 1:1, 1:2, 2:1 and 2:2 of lawsone and soya lecithin were prepared

by the antisolvent precipitation technique as mentioned in **Table 1**.

TABLE 1: PREPARATION OF PHYTOSOME COMPLEXES OF LAWSONE

Phytosomes	Phytosome (Molar ratio)	Drug	Phospholipid	Solvents
F1	1:1	Lawsone	Soya lecithin	Dichloromethane + n-hexane
F2	1:2	Lawsone	Soya lecithin	Dichloromethane + n-hexane
F3	2:1	Lawsone	Soya lecithin	Dichloromethane + n-hexane
F4	2:2	Lawsone	Soya lecithin	Dichloromethane + n-hexane

Powdered complex was placed in amber colored glass bottle and stored at room temperature.

Evaluation of phytosome complexes of lawsone:

1. Determination of % yield:

Determination of % yield of phytosome complex was calculated by the following formula:

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

2. Determination of particle size:
The average diameter of the lawsone-phospholipid complex was measured using a Nanophox (Sympatec GmbH, Germany) at a fixed scattering angle of 90° at 25^oC [15].

3. Determination of entrapment efficiency:
Phytosome complex of lawsone was diluted 1-fold with 10 ml of methanol and then centrifuged at 18,000 rpm for 1/2 h at -4^oC using cooling centrifuge machine. The supernatant was isolated and the amount of free lawsone was determined by UV/Vis spectroscopy at 269 nm. To determine the total amount of lawsone, 0.1 ml of the lawsone phospholipid suspension was diluted in methanol, adjusting the volume to 10 ml.

The Entrapment efficiency was calculated according to the following formula: [15-16].

Entrapment efficiency (%) =

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

4. Determination of drug content:
Drug content of phytosome complex was determined by dissolving accurately weighed 100 mg of complex in 10 ml methanol. After suitable dilution absorbance was determined by UV – Spectrophotometer at 269 nm and drug content was determined [15].

5. Scanning electron microscopy (SEM):
Scanning electron microscopy has been used to determine particle size distribution and surface morphology of the complexes. Samples were studied using JEOL JSM-6360 Scanning microscope (Japan). Dry samples were placed on an electron microscope brass stub and coated with gold in an ion sputter. Digital images of phytosome complex of lawsone were taken by random scanning of the stub at 1000, 5000, 10000 and 30000 X magnifications [15-17].

6. Differential scanning calorimetry (DSC):
Lawsone, soya lecithin phospholipids, physical mixture of lawsone and soya lecithin and phytosome complex of lawsone were placed in the aluminum crimp cell and heated at 10^oC/min from 0 to 400^oC in the atmosphere of nitrogen (TA Instruments, USA, model DSC Q10 V24.4 Build 116). Peak transition onset temperatures were recorded by means of an analyzer [15-17].

7. FTIR Spectroscopy:
FTIR (Perkin Elmer, Spectrum BX, USA) spectral data were taken to ascertain the structure and chemical stability of phytosome complex, soya lecithin phospholipids and plant drug lawsone. Samples were crushed with KBr to get pellets at 600 kg/cm^2 pressure. Spectral scanning was done in the range between 4000- 400 cm^{-1} [15-17].

8. Antifungal Activity:
The *in-vitro* antifungal activity by agar well diffusion method was standardized using ketoconazole. This method is based on diffusion of antifungal component from reservoir hole to the surrounding inoculated Sabouraud's dextrose agar medium, so that the growth of fungus is inhibited

as zone around the hole. Fungi *Candida albicans* (NCIM 3471) was selected. Sabouraud's dextrose agar medium was used for preliminary antifungal activity. The medium was prepared by dissolving in water and autoclaving at 121°C for 15 minutes. The pH of the medium plays an important role for the growth of fungi. Acidic medium favour the growth but excess of acid prevent solidification of agar. Hence the pH of medium was adjusted using 0.1%w/v lactic acid. Ketoconazole standard was prepared at a final concentration of 10 mg/ml of sterile distilled water.

The suspension of fungus was prepared as per Mac-Farland Nephlometer standard. A 24 hour old culture was used for the preparation of fungus suspension. A suspension of fungus was made in a sterile isotonic solution of sodium chloride and the turbidity was adjusted such that it contained approximately 1.5×10^{6} cells/ml [18].

Working procedure:
An inoculum was prepared by suspending a single isolated colony in about 5 ml of 0.9 % w/v of normal saline. This is mixed slowly to achieve a smooth suspension. Later on, the mould was broken by shaking. The test tube was tightly closed with a sterile cotton plug.

The sterile hot sabouraud's agar medium (30 ml) was poured in each plate and allowed to harden on a level surface. A glass spreader was moistened in the adjusted inoculums suspension of fungi *Candida albicans* and streaked to cover the entire surfaces of sabouraud's agar plates. Using flamed sterile borer the medium was bored and the prepared sample of 10 mg/ml concentrations was taken and 0.1 ml of samples was added in each bore. This procedure was carried out for the following:

1. Plant drug lawsone
2. Phytosome complex 1:1 (F1)
3. Phytosome complex 1:2 (F2)
4. Standard drug ketoconazole

The above operation was carried out in aseptic condition. A control having only sterile distilled water was maintained in each plate. The plates are incubated at 28°C for 24 hr. Later the values of zones of inhibition were recorded.

Preparation of gels of phytosome complex and plant drug:
The gels of the phytosome complexes and plant drug lawsone were prepared by using different excipients and solvents as mentioned in **Table 2**.

TABLE 2: PREPARATION OF GELS OF PHYTOSOME COMPLEX AND PLANT DRUG

Name of Ingredients	Formulation of Drug gel		Formulation of Complex gel of 1:1		Formulation of Complex gel of 1:2	
	G1	G2	G3	G4	G5	G6
Carbopol® 934P	1 %	1 %	1 %	1 %	1 %	1 %
PEG 400	2.5 %	2.5 %	2.5 %	2.5 %	2.5 %	2.5 %
Drug/complex	1%	2%	1%	2%	1%	2%
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Methyl Paraben	0.1 %	0.1 %	0.1 %	0.1 %	0.1 %	0.1 %
Propyl Paraben	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
Ethanol	1 %	1 %	1 %	1 %	1 %	1 %
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s

The required amount of methyl and propyl paraben were dissolved in 10 ml of distilled water in a beaker to which Carbopol® 934P was dispersed and stirred continuously. The solution of lawsone / phytosome complex was prepared in 0.1 ml of ethanol in another beaker and was added to the Carbopol dispersion. PEG 400 was also added to the dispersion. Triethanolamine was added to the dispersion to form gels. Prepared gels were filled in Al-tubes and stored at room temperature for further studies.

Evaluation of gels of phytosome complex and plant drug lawsone:

1. pH measurement:
The pH of the phytosome gels were measured with the help of digital pH meter. 0.5 g of phytosome gel was dissolved in 50 ml of distilled water and stored

for two hrs. The measurement of pH of each formulation was determined [15, 19-21].

2. Drug content:
Drug content of phytosome gels were determined by dissolving accurately weighed 100 mg of gels in 10 ml methanol. After suitable dilution absorbance was determined by UV – Spectrophotometer at 269 nm and drug content was determined [15, 19-21].

3. Uniformity:
All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container.

4. Spreadability:
The spreadability of phytosome gels were determined by placing 0.5 gm of gel on a glass slide within a circle of 1 cm. Place another glass slide on it and rest 500 gm of weight on upper slide. Increase in diameter due to spreading of gel was determined [15, 19-21].

5. *In vitro* permeation study:
The phytosome complex gel of lawsone measuring 1.5 cm^2 was subjected to an *ex vivo* permeation study using a modified Franz diffusion cell (cell capacity 7 ml). Based on literature survey, phosphate buffer saline pH 7.4 was used as a diffusion media. The phytosome complex gel of lawsone and plant drug gel was applied on the dermal surface of 1.5 cm^2 cellulose acetate membrane/rat skin. The diffusion media was continuously stirred with needle shaped magnetic stirrer rotating at a speed of around 300-350 rpm. The temperature was maintained at 32 ± 0.5°C with the help of circulating hot water. The diffusion was carried out for 6 hours. At pre-determined time intervals (0.5, 1, 2, 4 and 6 hours), 0.5 ml sample were withdrawn and replaced with the same volume of fresh phosphate buffer saline having pH 7.4. Absorbance of the solutions was measured UV spectrophotometerically at 227 nm. The cumulative percentage drug permeation of the phytosome complex gel of lawsone and plant drug gel were determined [22-23].

6. *In vivo* anti - inflammatory study:
Male wistar rats were divided into four groups such as control, inflammation, phytosome gel and plant drug gel groups. All rats were fed standard rat chow and were maintained on a 12-hour light/dark cycle.

In addition, rats were acclimated to the procedure of anti-inflammatory activity measurement daily for 1 week. Inflammation was induced in rats by injecting, 0.5 ml of carrageenan (1% w/v) underneath the plantar region of the right and left paw. Anti-inflammatory activity was measured by digital plethysmometer (PM 01 Orchid Scientifics, India) [24-25].

RESULT AND DISCUSSION:

The qualitative results obtained from various methods are shown in **Table 3.**

TABLE 3: EVALUATION OF PRELIMINARY BATCHES FOR SELECTION OF WORKING METHOD

Methods	% Yield	Particle size (nm)	% Entrapment Efficiency
Antisolvent precipitation	87.85	751.46	95.6 ± 0.7
Rotary evaporation technique	73.19	254.88	82.1 ± 0.3
Solvent evaporation	70.63	448.91	70.3 ± 0.9

It was found that the antisolvent precipitation method showed 751 nm particle size, 95.6±0.7 % entrapment efficiency and 88% yield as compared to 254 nm particle size, 82.1± 0.3 % entrapment efficiency, 73% yield by rotary evaporation technique and 448 nm particle size, 70% entrapment efficiency, 70.3±0.9 % yield by solvent evaporation. Phytosome complex of lawsone 1:1 molar ratio by antisolvent precipitation technique showed better % yield, particle size and % entrapment efficiency as compared to other methods. Thus, the antisolvent precipitation technique was selected for the preparation of final formulations of phytosome complex of lawsone for further studies. The phytosome complexes of lawsone F1 to F4 were evaluated and are shown in **Table 4.**

TABLE 4: EVALUATION OF PHYTOSOME COMPLEXES OF LAWSONE

Phytosomes	% Yield	Particle size (nm)	% Entrapment Efficiency	% Drug Content
F1	91.10	779.89	97.7 ± 0.5	90.1
F2	84.34	527.90	94.3 ± 0.8	85.8
F3	74.56	268.88	92.3 ± 0.2	81.3
F4	72.08	22.53	90.0 ± 0.2	72.7

The phytosome complexes of lawsone F1 to F4 showed 91.10%, 84.34%, 74.56%, and 72.08% yield respectively. The drug content was found to be 90.1%, 85.8%, 81.3% and 72.7% of phytosome complexes of lawsone F1 to F4 respectively. The particle size varies from 22.53 nm to 779.89 nm. It was found that phytosome complex of lawsone F1 to F4 showed 779.89 nm, 527.90 nm, 268.88 nm and 22.53 nm particle sizes respectively. The entrapment efficiency of phytosome complex of lawsone varies from 90.09 ± 0.23 to 97.7 ± 0.50. It was found that phytosome complex of lawsone F1 to F4 showed 97.7 ± 0.50 %, 92.3 ± 0.2 %, 94.3 ± 0.8 %, and 90.09 ± 0.23 % entrapment efficiency respectively. Phytosome complex of lawsone F1 and F2 showed better % yield, drug content, particle size and entrapment efficiency as compared to other phytosome complexes F3 and F4. Therefore F1 and F2 were used for further studies. FTIR studies showed that the peaks of drug and soya lecithin were not seen in the spectrum of phytosome F1 so it confirmed the formation of phytosome complex of lawsone with soya lecithin as shown in **Fig.1**.

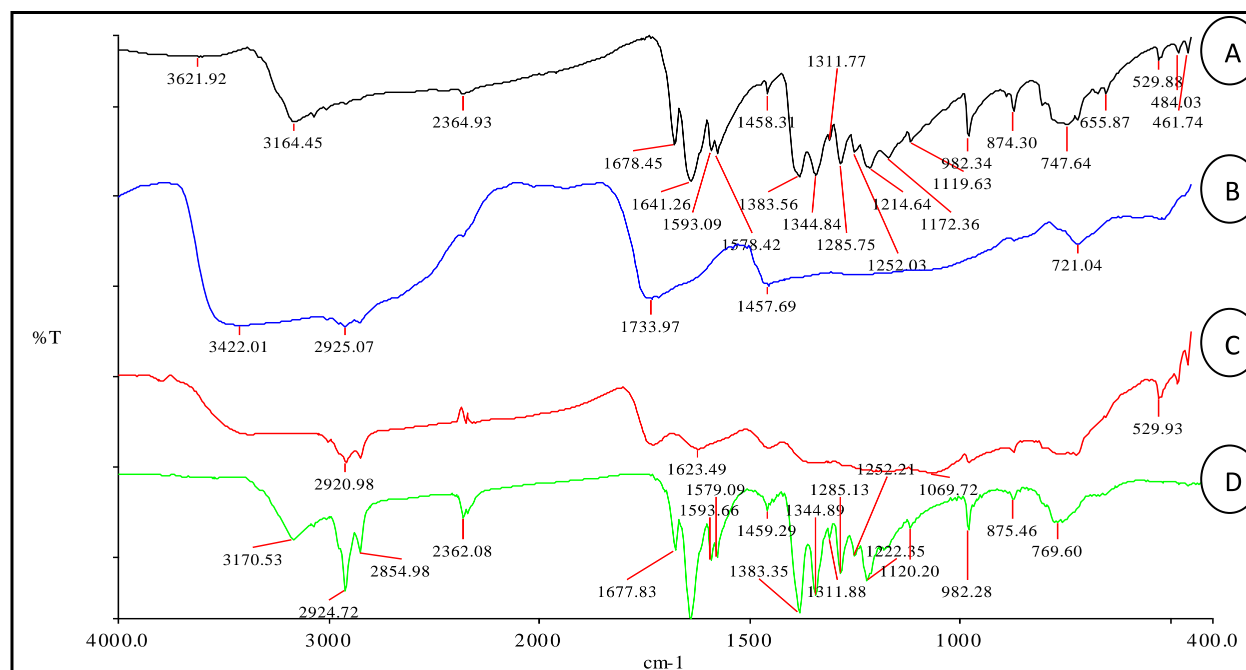

FIG.1: IR SPECTRUM OF LAWSONE (A), SOYA LECITHIN (B), PHYSICAL MIXTURE (C) AND PHYTOSOME F1 (D)

In DSC studies, Lawsone showed a broad endothermal peak and its beginning melting point was 198°C. Soya lecithin showed an endothermal peak at 157.1°C. However, the endothermal peak at 157.1°C appears sharp-pointed. It was considered that owing to the transition from gel state to liquid crystal state, the carbon-hydrogen chain in soya lecithin perhaps happened to be melt, isomerous or the crystal changes. The physical mixture of lawsone and soya lecithin showed two major peaks at 154.03°C and 267.50°C. The phytosome F1 showed peak at 273.03 °C which is higher than the physical mixture. The slight increment in temperature might be due to strong interaction between lawsone and soya lecithin. The DSC of phytosome F1 showed peaks of drug and phospholipids disappeared and the phase transition temperature of phospholipids. So the lawsone and soya lecithin must have some interaction, such as the combination of hydrogen bonds or vander Waals force. The results are depicted in **Fig. 2**.

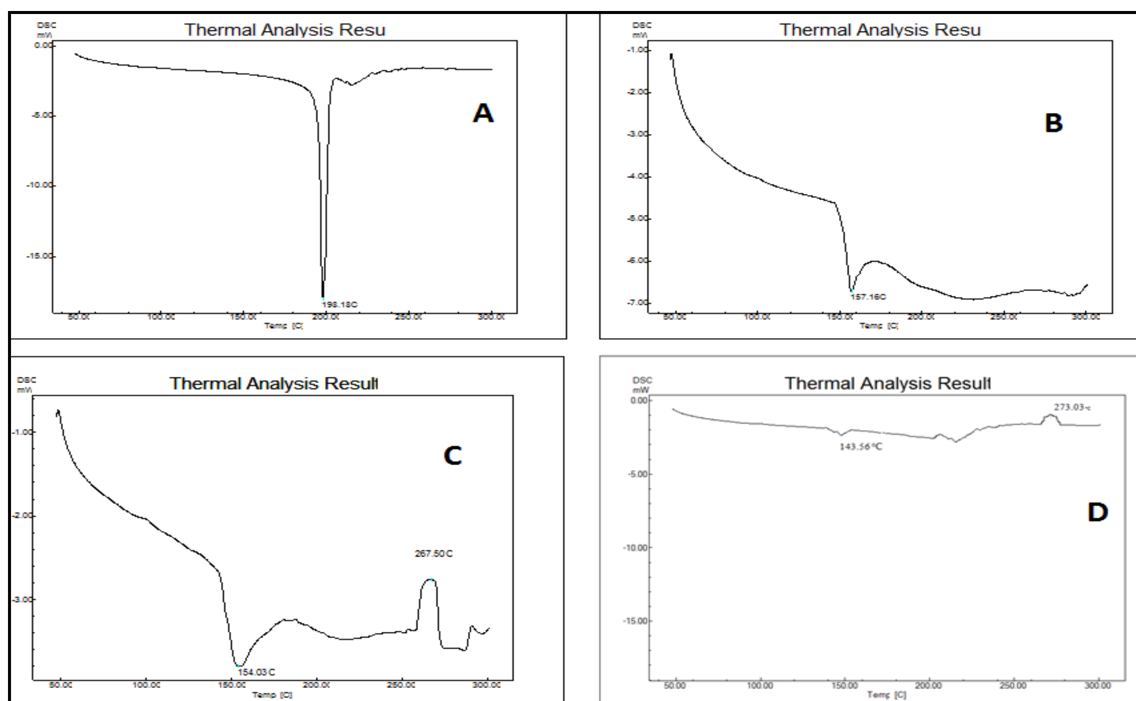

FIG. 2: DSC THERMOGRAM OF LAWSONE (A) SOYA LECITHIN (B) PHYSICAL MIXTURE (C) PHYTOSOME F1 (D)

The surface morphology, shape and structure of the phytosome complex of lawsone F1 at various magnifications are shown in **Fig.3**. It was observed that the drug particles are associated with the phospholipid forming complexes with irregular particles shape and crystalline structures.

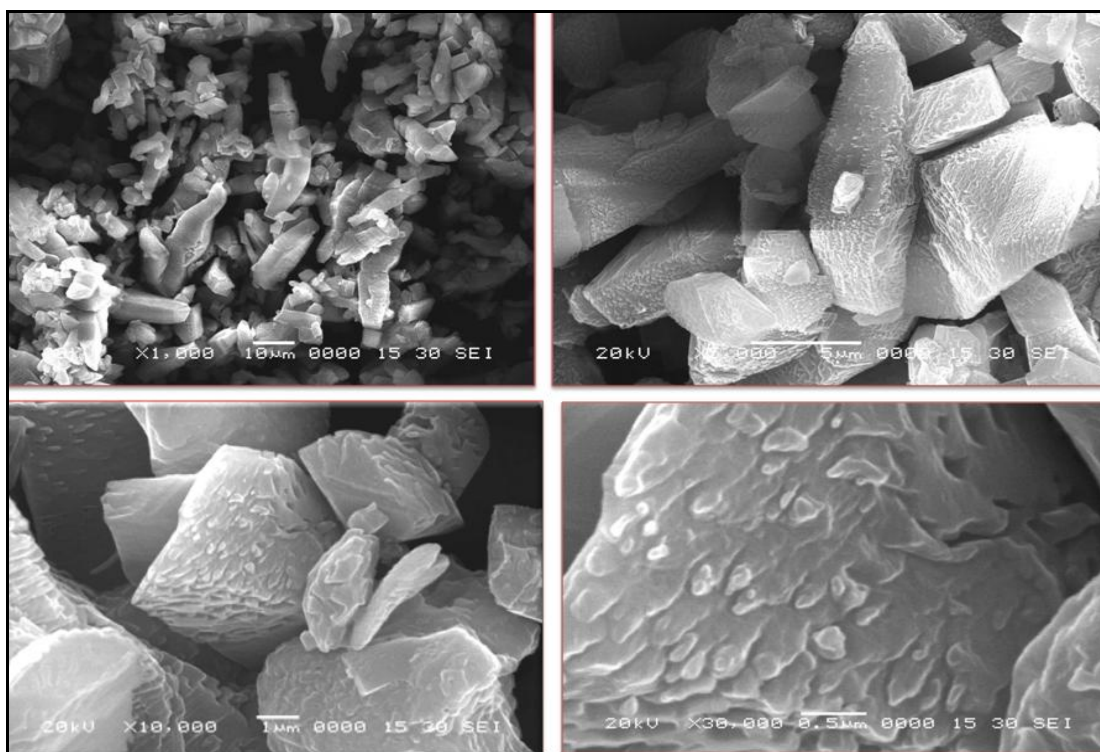

FIG. 3: SURFACE MORPHOLOGY AND PARTICLE SIZE DISTRIBUTION OF PHYTOSOME F1 BY (SEM) AT (A) 1000X (B) 5000 (C) 10000X AND (D) 30000X MAGNIFICATIONS

Phytosome complexes of lawsone F1 and F2 were evaluated for antifungal activity. The antifungal activity of phytosome complex of lawsone is shown in **Table 5**.

TABLE 5: ANTIFUNGAL ACTIVITY OF PLANT DRUG, COMPLEX 1:1 AND STANDARD DRUG

Days	Zone of inhibition (mm)			
	Plant drug Lawsone	**Complex 1:1 F1**	**Complex 1:1 F2**	**Standard Ketoconazole**
1	21 ± 0.5	24 ± 0.04	20 ± 0.1	20 ± 0.02
2	18 ± 0.07	23 ± 0.2	18 ± 0.03	18 ± 0.08
3	17 ± 0.01	22 ± 0.09	17 ± 0.06	16 ± 0.3
Averages =	18 ± 0.58	23 ± 0.39	19 ± 0.19	18 ± 0.4

It was found that phytosome complex F1 showed 23 ± 0.39 mm zone of inhibition as compared to 19 ± 0.19 mm, 18 ± 0.58 mm and 18 ± 0.40 mm zone of inhibition of phytosome complex F2, plant drug and standard drug ketoconazole after 3 days. The phytosome complex of lawsone F1 showed better antifungal activity as compared to phytosome complex F2, plant drug and standard drug. The gels of plant drug lawsone G1 & G2 and phytosome complex of lawsone G3 – G6 were evaluated and are shown in **Table 6**.

TABLE 6: EVALUATION OF GELS OF PHYTOSOME COMPLEX AND PLANT DRUG

Formulation gels	Physical observation	pH	% Drug Content	Uniformity	Spreadability Increase in diameter (cm)
Drug 1% (G1)	Orange red color, greasy homogenous	6.9 ± 0.04	92.97 ± 0.08	Good	5.2 ± 0.03
Drug 2% (G2)	Orange red color, greasy homogenous	7.0 ± 0.12	93.90 ± 0.25	Good	5.9 ± 0.06
Complex 1:1 1% (G3)	Orange red color, greasy homogenous	7.1 ± 0.20	95.93 ± 0.14	Good	6.2 ± 0.04
Complex 1:1 2% (G4)	Orange red color, greasy homogenous	7.0 ± 0.06	98.89 ± 0.03	Good	6.6 ± 0.07
Complex 1:2 1% (G5)	Orange red color, greasy homogenous	7.2 ± 0.32	96.15 ± 0.09	Good	6.1 ± 0.09
Complex 1:2 2% (G6)	Orange red color, greasy homogenous	7.1 ± 0.10	97.02 ± 0.45	Good	6.3 ± 0.01

The pH of all gels was found to between the ranges of 6.9 to 7.2. The formulation G1 & G2 of plant drug gels showed 92.97 ± 0.1 %, 93.90 ± 0.73 % drug content respectively. Phytosome gels G3 & G4 showed 95.93 ± 0.23% & 98.89% ± 0.02% and G5 & G6 complex gels showed 96.15 ± 0.03 %, 97.02 ± 0.4 % drug content respectively. The spreadability of all gels was found to be between the ranges of 5.2 cm to 6.6 cm. The formulation G1 & G2 of plant drug gels showed spreadability of 5.2 cm and 5.9 cm as compared to phytosome gels G3 & G4 which showed good spreadability of 6.2 cm and 6.6 cm. G5 & G6 complex gels showed 6.1 cm and 6.3 cm spreadability. Thus, phytosome gels of complex of lawsone formulation G4 showed better drug content, uniformity and spreadability as compared to other gels. *Ex vivo* permeation studies through rat skin of formulation G1-G6 are shown in **Fig. 4**.

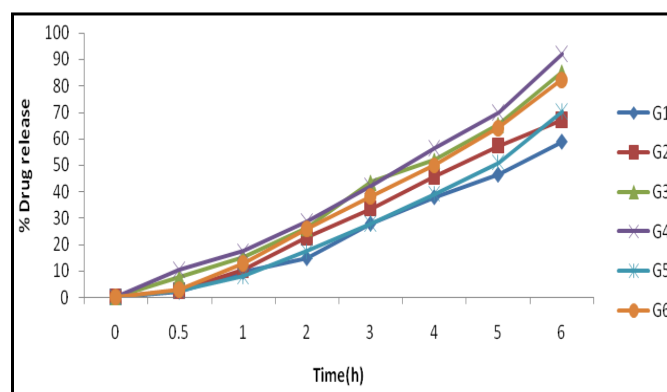

FIG.4: *EX-VIVO* PERMEATION STUDY THROUGH RAT SKIN

Formulation G1 and G2 containing 1% and 2% plain drug lawsone showed 58.92%, 69.33% of cumulative percentage drug permeation up to 6 hours. Formulation G3 and G4 containing 1% and 2% of complex of 1:1 molar ratio of lawsone showed 85.79%, 92.91% of cumulative percentage drug permeation up to 6 hours. Formulation G5 and G6 containing 1% and 2% of complex of 1:2 molar ratio of lawsone showed 70.51%, 82.43% of cumulative percentage drug permeation up to 6 hours. Formulation G4 showed best percentage of cumulative drug permeation through rat skin up to 6 hours than other formulation.

Anti-inflammatory activity studies of phytosome complex gel G4 and plant drug gel are shown in Figure 5.

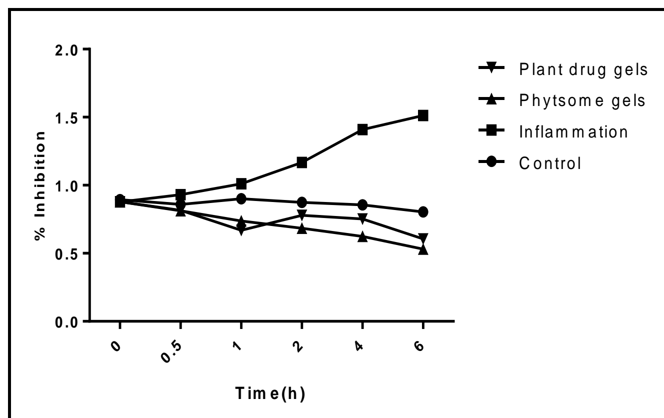

FIG.5: *IN-VIVO* ANTI-INFLAMMATORY ACTIVITY BY CARRAGEENAN INDUCED RAT PAW OEDEMA
Values are expressed mean ± SEM, n=6. ***P<0.001 compared with complex gels and plant drug gels.

It showed 96.33 ± 0.34% and 81.53 ± 0.23 % of inhibition respectively at the end of 6 h. The phytosome gel G4 containing phytosome complex showed significant anti-inflammatory activity as compared to plant drug gel at 4 h ($P < 0.001$).

CONCLUSION: Based on the above study it can be concluded that the phytosome complex of lawsone F1 showed better antifungal activity as compared to phytosome complex of lawsone F2 and plant drug lawsone & standard drug ketoconazole. The phytosome gels of complex of lawsone formulation G4 showed better drug content, uniformity and spreadability as compared to other gels.The gel G4 containing 2% of 1:1 ratio of phytosome complex of lawsone is the best formulation among all formulations on the basis of its permeation studies. The gel of phytosome complex of lawsone G4 showed better percentage of cumulative drug permeation as compared to plant drug gel through rat skin up to 6 hours. The gel G4 containing phytosome complex of lawsone showed significant anti-inflammatory activity as compared to plant drug gel formulation at 4 h ($P < 0.001$).

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