

## Preparation and Evaluation of Phytosome of Herbal Plant of *Lawsonia inermis L* for Topical Application

Rudra Pratap Singh Rajput<sup>1</sup>, Chinmoyi Chakravarty<sup>2</sup> and Shiv kumar Bhardwaj<sup>3</sup>

<sup>1</sup>Rudra Pratap Singh Rajput, Columbia Institute of Pharmacy, Raipur,

<sup>2</sup>Chinmoyi Chakravarty, Jeypore College of Pharmacy, Jeypore, Odisha, India

<sup>3</sup>ShivBhardwaj, Columbia Institute of Pharmacy, Raipur

<sup>1</sup>rudra.p.s007@gmail.com

**Abstract:** *Lawsonia inermis L.* (Lawsonia) was reported to contain carbohydrates, proteins, flavonoids and phenolic compounds, alkaloids, terpenoids. The aim of this study was to prepare and evaluate the phytosome containing lawsonia. The phytosomes (P1 – P4) containing different molar ratios (1:1, 1:2, 2:1 and 2:2) of lawsonia and soya lecithin were prepared by the antisolvent precipitation technique. The phytosomes were evaluated for % yield, particle size analysis, % EE and characterized by FTIR, DSC and SEM. Antifungal activity of phytosome of lawsonia was evaluated on *Candida albicans* (NCIM 3471) fungi by using ketoconazole as standard drug. The *in-vitro* permeation study was carried out on rat skin. The anti-inflammatory activity was evaluated in male wistar rats.

The phytosomes of lawsonia P1 and P2 showed better % yield, drug content, particle size and entrapment efficiency as compared to other phytosomes P3 and P4. The infra-red (FT-IR) and Differential Scanning Calorimetry (DSC) studies of phytosome of lawsonia revealed that there was no interaction between the plant drug and phospholipids. SEM data showed that phytosome of lawsonia P1 has irregular size vesicles consisting of soya lecithin and it was found to be intercalated in the lipid layer. Antifungal activity of phytosome P1 (1:1) showed the better zone of inhibition as compared to phytosome P2 (1:2), drug lawsonia and standard drug ketoconazole after 3 days. *In-vitro* permeation study of phytosome gel of lawsonia (PG) through excised rat skin showed 92.91% of drug permeation up to 6 h. The anti-inflammatory activity of gel of phytosome of lawsonia showed significant anti-inflammatory activity as compared to gel of drug lawsonia at 4 h ( $P < 0.001$ ).

**Keywords:** Phytosome, Lawsonia, Soya lecithin, Anti-solvent precipitation technique, *In vitro* study.

### 1. INTRODUCTION

*Lawsonia inermis L.* (Lawsonia) is a much branched glabrous herb. Lawsonia is used to treat painful conditions such as analgesic, hepatoprotective, and anti - inflammatory, antifungal and shows low bioavailability and rapidly eliminated from the body.

Phytosome is a patented technology, which improves the absorption and bioavailability of lipid compatible molecular complexes<sup>1</sup>.

## 2. EXPERIMENTAL METHODS

### 2.1. Preparation of phytosomes of lawsonia

Phytosomes of lawsonia were prepared by anti-solvent precipitation technique using lawsonia and soya lecithin. Phytosomes P1-P4 was prepared containing dichloromethane and n-hexane as solvents and molar ratio of 1:1, 1:2, 2:1 and 2:2 of lawsonia and soya lecithin. The dried precipitate was crushed in mortar and sieved through #100 meshes. Powdered phytosome was placed in amber colored glass bottle and stored at room temperature.

### 2.2 Evaluation of phytosomes of lawsonia

Evaluation of the phytosome was done for % yield, particle size, entrapment efficiency, drug content and Scanning electron microscopy (SEM).

### 2.3 Antifungal Activity

Agar well method was selected to standardize the *in-vitro* antifungal activity. *Candida albicans* (NCIM 3471) was used as fungi & an inoculum was prepared as per Mac-Farland Nephelometer standard. The sterile hot sabouraud's agar medium was prepared by dissolving in water and autoclaving at 121°C for 15 minutes and poured in each plate and allowed to harden on a level surface. This procedure was carried out for the different formulations. The plates are incubated at 28°C for 24 hr. Later the values of zones of inhibition were recorded.

### 2.4 Preparation of gels of phytosome and drug lawsonia

The gels of phytosomes and drug were prepared by using methyl and propyl paraben, Carbopol® 934P, PEG 400 and Triethanolamin. Prepared gels were filled in Al-tubes and stored at room temperature for further studies.

### 2.5 Evaluation of gels of phytosome and drug lawsonia

Evaluation of the prepared gel was done for physical appearance, pH measurement, drug content, uniformity and spread ability.

**(a) *In vitro* permeation study<sup>2</sup>:** Full skin was excised from the dorsal side of dead Wistar rat and the skin was washed with physiological saline solution. For drug permeation study phosphate buffer saline pH 7.4 was used as a diffusion media. The diffusion was carried out at pre-determined time intervals (0.5, 1, 2, 4 and 6 hours) 0.5 ml sample was withdrawn and replaced with the same volume of fresh phosphate buffer solution having pH 7.4 and analyzed spectrophotometrically.

**(b) *In vivo* anti - inflammatory study<sup>3</sup>:** Male Wistar rats, initially weighing 180-200 gm, were used for experiment. Rats were divided into four groups. Inflammation was induced in rats by injecting, 0.5 ml of Carrageenan (1% w/v) underneath the plantar region of right and left paw. Anti-inflammatory activity was measured by digital plethysmometer (PM 01 Orchid Scientifics, India). The percent inhibition in hind paw edema volume was calculated using the following formula and compared with those recorded for control group. ( )

Where, D is the change in paw volume in the treated group and C is the change in paw volume in the control group

### 3. RESULTS AND DISCUSSION

The phytosomes of lawsone P1-P4 were prepared by anti-solvent precipitation technique and after the evaluation, the % yield was found to be 91.10%, 84.34%, 74.56%, and 72.08% yield. The Particle size was between 22.53 to 779.89 nm. The % EE was between  $90.0 \pm 0.2$  to  $97.7 \pm 0.5$  and the % drug content was between 72.7 to 90.1%. The Phytosomes of lawsone P1 and P2 showed better % yield, drug content, particle size and entrapment efficiency as compared to other phytosomes F3 and F4. Therefore P1 and P2 were used for further studies. The antifungal activity of phytosome P1 was found to be  $23 \pm 0.39$  mm as compared to  $19 \pm 0.19$  mm,  $18 \pm 0.58$  mm and  $18 \pm 0.40$  mm zone inhibitions of phytosome P2, drug lawsone and standard drug ketoconazole after 3 days. The gels of drug lawsone G1 & G2 and phytosome complex of lawsone G3-G6 were prepared and evaluated. The pH of all gels was found to be between the ranges of 6.9 to 7.2. The % drug content of gel formulations G1- G6 varied from  $92.97 \pm 0.1$  to  $97.02 \pm 0.4$  %. The spread ability of all gels was found to be between the ranges of 5.2 cm to 6.6 cm.

The surface morphology, shape and structure of the phytosome of lawsone P1 at various magnifications were observed that the drug particles are associated with the phospholipid forming complexes with irregular particles shape and crystalline structures. *Ex vivo* permeation study of optimized gel formulation G4 showed higher % drug permeation of 92.91% as compare to all other gel formulations and also drug gel formulations.

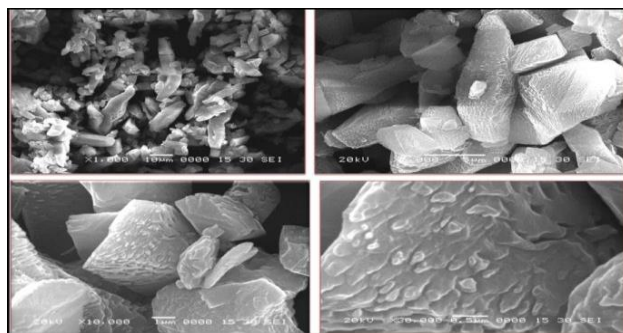


Figure 1. Surface morphology and particle size distribution of phytosome by SEM.

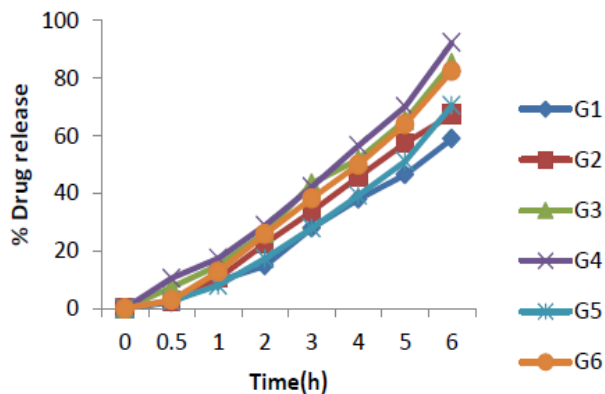


Figure 2. *Ex- vivo* Permeation study through rat skin.

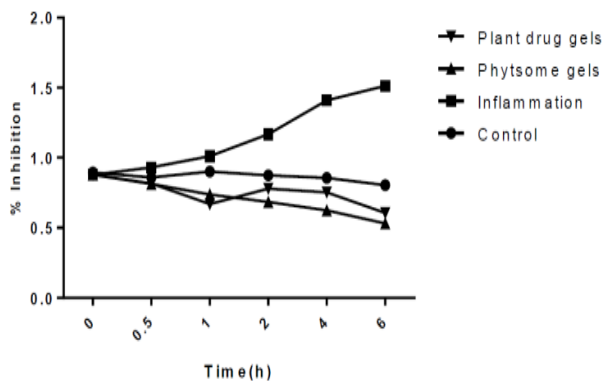


Figure 3: Optimized gel formulation G4 containing 2% of complex of 1:1 molar ratio of lawsone

Optimized gel formulation G4 containing 2% of complex of 1:1 molar ratio of lawsone showed significant anti-inflammatory activity as  $96.33 \pm 0.34$  % of inhibition of inflammation as compared to drug gel formulation containing 2% lawsone  $81.53 \pm 0.23$  % at 4 h ( $P < 0.001$ ).

#### 4. CONCLUSION

The antifungal activity, permeation rate activity and anti – inflammatory activity of phytosome of lawsone were found to be better than drug lawsone. Thus, a phytosome was successfully prepared using lawsone and soya lecithin.

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