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Formulation and Evaluation of Anti-dandruff Hair Gel containing Lawsone

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Abstract

Lawsone is the principle colouring compound of Henna, *Lawsonia inermis* Linn. (Fam. Lythraceae). Henna has been used to treat skin infections such as tinea and it is known to have antibacterial and antifungal properties which have been attributed to naphthoquinones, including lawsone.Anti-dandruff activity of lawsone was determined on the pure culture of *Malassezia Furfur* at different concentration. Lawsone showed good antidandruff activity. Lawsone gel was prepared by using carbopol 940 as a polymer in varying concentration from 0.5g-3g. After preparation of formulation the formulation were evaluated for drug content, viscosity and spreadability. In-vitro diffusion of lawsone hair gel was performed in phosphate buffer 5.0. Formulation batch L2 showed 94.20% cumulative percentage drug release up to 6hr. Same formulations were evaluated for the zone of inhibition for their anti-dandruff activity against *Malassezia Furfur.* The formulations L2 batch shows good zone of inhibition with compared to clotrimazole gel.

Keywords: Lawsone, dandruff, Carbopol 940, hair-gel,

1. INTRODUCTION

Dandruff is a very common non-contagious hair problem, nearly affecting person irrespective of age. Medically it is defined as pityriasis simplex capitis – shedding of dead skin from the scalp. It may be dry or greasy, dry dandruff appears silvery and white while greasy flakes appear pale yellowish and may have an unpleasant smell [1]. Historically there have been multiple other descriptive names reflecting the fungal cause of this condition, such as pityriasis simplex and pityriasis capitis (referring to Pityrosporum) and furfuracea (referring to *Malassezia furfur*) [2,3]. It is a common embarrassing disorder which effects 5% of the global population [4, 5]. Dandruff affects the aesthetic value and causes the itching and keratinocytes play major role in the expressions and the generation of immunological reaction during dandruff formation [6, 7]. The severity of dandruff may fluctuate with season and often worsen in winter. Dandruff is common scalp condition that produces the irritating white flakes and itchy scalp,excessive drying of skin and over-activity of oil gland known as seborrhea [8,9].

Lawsone, a red-orange coloured compound is the principle colouring compound of Henna, *Lawsonia inermis* Linn. (Fam. Lythraceae) (Figure 1).

Chemically, it is called as 2-hydroxynaphthalene-1,4-dione having molecular formula $C_{10}H_6O_3$, molecular weight 174.15 g/mol and melting point 195-196 °C [10]. Henna has been used to treat skin infections such as tinea and it is known to have antibacterial and antifungal properties which have been attributed to naphthoquinones, including lawsone [11].

Currently available treatment options for the management of Seborrhoeic Dermatitis include therapeutic use of Selenium sulphide, zinc pyrithione, salicylic acid, imidazole derivatives, glycolic acid, steroids, and coal tar derivatives. However, these agents show certain limitations, either due to poor clinical efficacy or due to the compliance issues. Futhermore, these drugs are unable to prevent recurrence.

Therefore, to overcome all these side effects an attempt been made to formulate and evaluate antidandruff gel containing Lawsone to minimize all these side effects and to show rapid action on Dandruff.

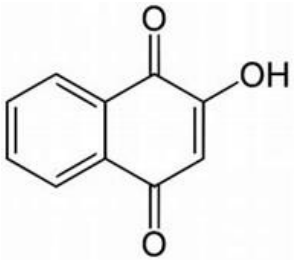

Figure 1: Structure of lawsone

2. MATERIALS AND METHODS

2.1 Materials

Pure Lawsone was purchased from Sigma Aldrich, India. Carbopol 940, Glycerine, Cellophane Membrane 150 and DMSO were procured from Analab Fine Chem, Mumbai. Clotrimazole was procured from EmcurePharma. Pvt.Ltd., Pune. Methyl Parabenand Sabouraud dextrose agar media were obtained from Hi-media Lab.The strain *Malassezia furfur (*MTCC1374) was obtained from Microbial Culture Collection, Chandigarh, India.

2.2 Anti-dandruff activity of lawsone

The antidandruff activity of lawsone was determined by cup plate method. The hot Sabouraud dextrose media was poured into sterilised petri dishes to give a depth of 3-4 mm under sterile condition using laminar flow unit. The uniform layer of medium was spread in petridish. After solidification the plates were dried for 30 min in an incubator to remove excess moisture from the surface. Subculture of *Malassezia Furfur* was added onto the surface of solidified media and was spread uniformly with

the help of sterile spreader. After stabilization of culture, with the help of a sterile cork borer, cups of each 6 mm diameter were punched and scooped out from the petridish. Different concentrations of lawsone and clotrimazole such as 200, 400, 600, 800 and 1000 µg/ml were dissolved in dimethylsulfoxide and fed into the well. The petridishes were then incubated for 48 hrs at 37 °C. After incubation the zone of inhibition was measured using zone reader [12].

2.3 Determination of minimum inhibitory concentration

Nutrient broth (double strength) test tubes were prepared and labelled. The first tube a (UT), was uninoculuated acted as negative control. Tube b (CT) was control (inoculum added in it, without lawsone). In tubes c, d, e, f, drug was added in concentration of 200, 400, 600, 800, 1000 µg/ml with dimethylsulfoxide respectively. Inoculum (3-4 drops) was added to all tubes to get the final concentration of microorganisms of 10^6 cells/ml. Drug was added in all test tubes except uninoculuated (negative control) and control (positive) tube. The positive control tube (CT) was used to check the suitability of the medium for growth of the *Malassezia Furfur.* All test tubes were shaken and then incubated at 37^oC for 48 h All experiments were repeated three times and the results were expressed as average values [13].

2.4 Preparation of gel

Measured quantity of methylparaben, polyethylene glycol and glycerin were dissolved in 10ml of water in beaker and were stirred at high speed using mechanical stirrer. Then carbopol 940 was added slowly to the above beaker while stirring and it was allowed to hydrate for 2 hr (Phase I). Lawsone was dissolved in propylene glycol (Phase II) and then the Phase II solution was added slowly to the Phase I and stirred for 5 min. The final weight was adjusted to 20gm by adding sufficient amount of water with stirring. Finally Triethanolamine was added slowly while stirring till it attain neutralized gel structure.The details of formulations are shown in Table 1 [14,15].

2.5 Evaluation of Gel:

2.5.1 Physical evaluation:

Physical parameters such as color, appearance and consistency were checked visually.

2.5.2 pH of formulation:

The pH of the formulated gel was determined using digital pH meter (Systronics Instruments, India). The electrode was immersed in the gel and readings were recorded from pH meter [16].

2.5.3 Lawsone content:

Accurately weighed quantity of gel (100 mg) was dissolved 10 ml methanol, filtered and lawsone content was determined by analysing spectrophotometrically at 452nm [17].

2.5.4 Viscosity study:

The viscosity of prepared gel was measured by using Brookfield viscometer using a spindle No.63 at 100 rpm. Gel (50 g) was kept in 50 ml beaker which was set till spindle groove was dipped and dial reading was measured after three minutes. From the obtained reading, viscosity was calculated [18].

2.5.5 Spreadability:

The weighed quantity of gel (about 0.5g) was sandwiched between two glass slides. 100 g of weight was placed on the slides. The weight was placed for specific period of time for 10 min. Then weight was removed and diameter of the spread circle was measured at different points. Spreadiability was calculated by using formula [19].

$$S = (M \times L)/T$$

Where,

S is spreadability, M is weight placed on the slide, L is diameter of circle in cm and T is Time in Sec.

2.5.6 Texture profile analysis:

Texture profile analysis (TPA) of gel was performed using a CT3 Texture Analyzer (Brookfield) in compression mode by using spreadability accessory (TA-BT-KIT). Optimized gel formulation was filled into the female probe, taking care to avoid air pocket into the samples. A conical analytical male probe (35 mm diameter of 45°) was forced down into each sample at a defined rate (1 mm/s) and to a defined depth (10 mm). At least two replicate analysis of samples were performed [20].

2.5.7 In vitro permeation study*:*

Franz diffusion cell was used for the *in-vitro* permeation study of the gel formulation. Briefly, the gel was applied on the dermal surface of goat skin and the diffusion media (phosphate buffer saline pH 5.0, temperature 32 ± 0.5°C) was continuously stirred with magnetic stirrer for 6 hr. Sample (0.5 ml) were withdrawn at predetermined time intervals (0.5, 1, 2, 4 and 6 hr) and replaced with the same diffusion media. Lawsone content of the solutions was calculated by UV spectrophotometer at 452 nm and the cumulative percentage release of lawsone from the inclusion complex gel and lawsone gel were calculated [21,22].

2.5.8 Anti-dandruff activity of gel formulation:

In vitro anti-dandruff activity of formulated gel was performed using the well diffusion method. Suspension containing 3×10^8 CFU/ml of dandruff causing agent *Malassezia furfur* was used for the study. The anti-dandruff activity of lawsone gel was determined by cup plate method. The hot media was poured into sterilised petri dishes to give a depth of 3-4mm under sterile condition using laminar flow unit. 0.1 ml of culture suspension was spread evenly with the help of spreader and the wells were made aseptically with borer having 1.5 cm diameter. In each of these wells 1gm of gel was placed carefully. Plates were kept for pre diffusion for 30 minutes. After it normalized to room temperature ; the plates were incubated at 32˚C for 48hrs in incubator. After incubation period, the zone of inhibition was measured [23].

3. RESULTS AND DISCUSSION

3.1 Determination of anti-dandruff activity of lawsone:

In-vitro antidandruff activity of lawsone was performed against *Malassezia Furfur*. The cell density of the inoculum was adjusted with UV Visible

spectrophotometer in order to obtain a final concentration of approximately 10^8 colony forming units (optical density OD_{625} = 0.080.1). Mean zone of inhibition was calculated, which was taken as an indicator for the antidandruff activity of lawsone. Different concentrations of lawsone in 1ml DMSO(200- 1000 µg/ml of lawsone) showed good zone of inhibition (Table 2.). Increase in concentration of lawsone showed increase in zone of inhibiton. Thus, lawsone showed good antidandruff activity (Fig 2.).

Table 1: Composition of Different Gel Formulations

Ingredients	L1	L2	L3	L4	L5	L6
Lawsone (mg)	20	20	20	20	20	20
Carbopol 940 (g)	0.5	1	1.5	2	2.5	3
Propylene glycol (ml)	3	3	3	3	3	3
Polyethylene glycol-400 (ml)	4	4	4	4	4	4
Glycerin (ml)	0.6	0.6	0.6	0.6	0.6	0.6
Methyl paraben (g)	0.04	0.04	0.04	0.04	0.04	0.04
Triethanolamine (ml)	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s
Dist.Water (ml)	20	20	20	20	20	20

Table 2: Zone of inhibition of various concentrations

Sr. No.	Concentration (µg/ml)	Diameter of zone, (mm±SD), n=3
1.	200	18.73±0.058
2.	400	20.43±0.115
3.	600	23.20±0.000
4.	800	24.67±0.577
5.	1000	25.67±0.577

Table 3: Determination of MIC by broth diluton method

Tube number	Volume of double strength medium (ml)	Concentration of lawsone (µg/ml)	Visual results
(UT) a	5	0.0	Clear
(CT) b	5	0.0	Turbid
c	5	200	Turbid
d	5	400	Slightly Turbid
e	5	600	Clear
f	5	800	Clear

Table 4: Evaluation of lawsone gel formulation

Sr. No.	Formulation	pH	Viscosity (cps)	Appearance	Spreadability (gcm/sec)	Lawsone content
1	L1	6.8	55675	Transparent	1.09	91%
2	L2	7.2	51989	Transparent	1.02	94%
3	L3	6.9	56753	Transparent	0.77	95%
4	L4	6.4	57891	Transparent	0.5	91%
5	L5	6.9	58131	Transparent	0.51	94%
6	L6	6.5	58419	Transparent	0.45	92%

Table 5: Zone of inhibition of formulated gel with In house clotrimazole gel formulation

Formulation	Concentration (mg/ml)	Zone of Inhibition (mm)
Lawsone gel (L2)	1mg/ml	22.6±0.14
Clotrimazole Gel	1mg/ml	27.7±0.26

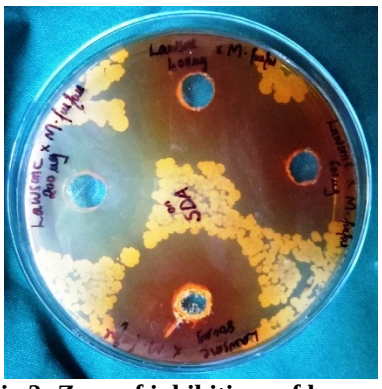

Fig 2: Zone of inhibitions of lawsone

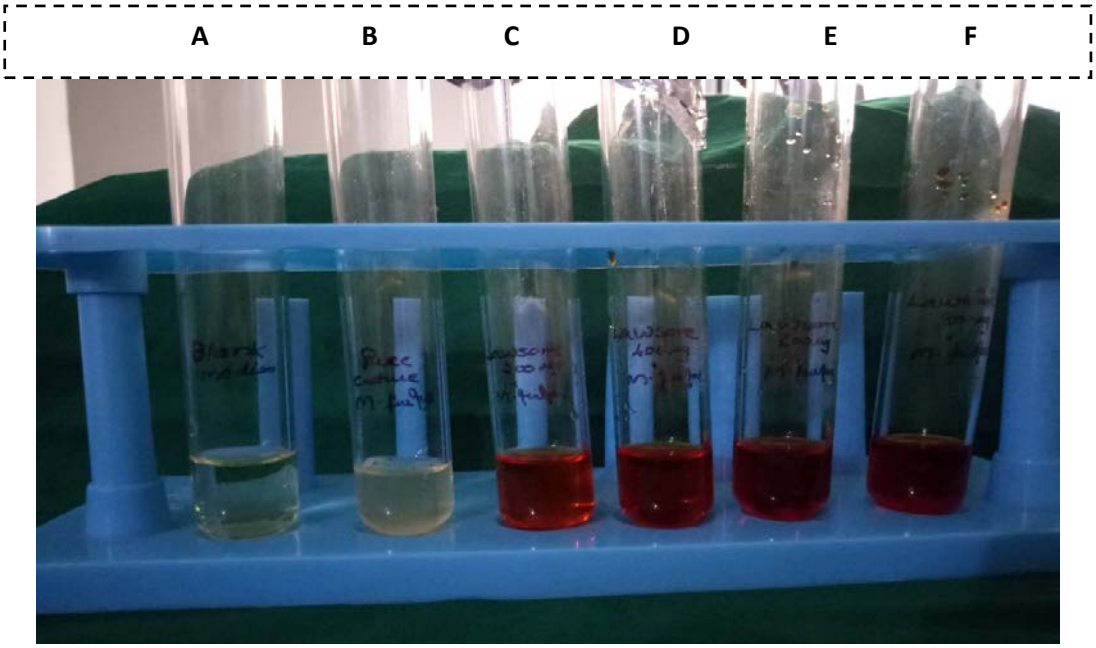

Fig 3: Determination of MIC by broth diluton method
A- UT (uninoculated), B- CT(control), C- 200g/ml, D- 400g/ml, E- 600g/ml, F- 800g/ml.

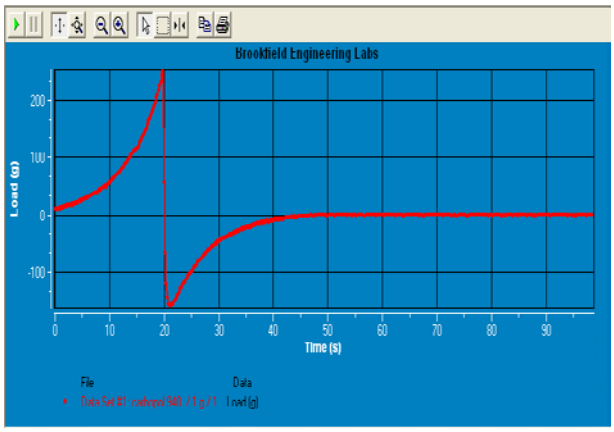

Figure 4: Texture profile analysis of formulated L2 optimized batch

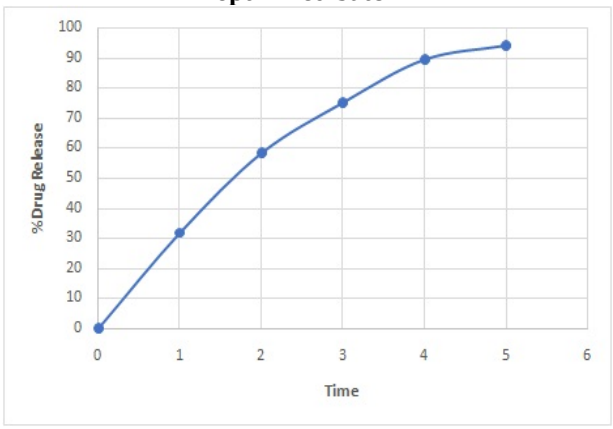

Fig. 5:*In-vitro* lawsone release from gel formulation

3.2 Determination of minimum inhibitory concentration:

Minimum inhibitory concentration is the lowest concentration of lawsone that inhibits the visible growth of *Malassezia Furfur*. The test tubes were named as UT (A) – uninoculated i.e., *Malassezia Furfur* was not inoculated in it and CT (B) was control. C (200 µg/ml) – showed full growth of *Malassezia Furfur*. D (400µg/ml) showed no remarkable killing of *Malassezia Furfur*. E and F (600 and 800µg/ml) showed remarkable clear media which indicated that the lawsone inhibited the growth of *Malassezia Furfur* present in test tubes. Hence, the lawsone showed antidandruff activity at MIC value 600 µg/ml which is nearly similar to the reported MIC of lawsone i.e., 512 µg /ml. The results are shown in Fig 3. and Table 3.

3.3 Preparation and Evaluation of gel:

The prepared gel formulations were orange transparent coloured, homogenous, and without air bubbles. All the formulations showed a pH in between 6-7 which is appropriate to prevent skin irritation.The spreadabilty of formulated gel was decreased as the concentration of gelling agent increased. Formulation L1 to L3 shows satisfactory spreadability. All formulation showed an increased viscosity as the concentration of the gelling agent was increased. By taking viscosity, spreadabiliy and pH in consideration of the formulation the L2 was taken as the optimized batch.

3.3.1 Determination of lawsone Content in gel formulations:

The content of lawsone was greater than 91% for all formulations, showing that the drug was distributed uniformly (Table 4).

3.3.2 Texture profile analysis (TPA) of gel:

Texture Profile Analysis spectra of gel of formulation L2 (Figure 4) showed the hardness (firmness) 365 g which is the maximum force value in graph. Area under the positive curve is the energy required to deform the sample (hardness work done) is 8.3 mJ, the hardness work done and firmness. This showed spreadability of sample. Higher

value of firmness and hardness work indicated less spreadable sample, conversely, the less value of same indicated more spreadable sample. The maximum negative force (210 g) on the graph indicated sample adhesive force ; the more negative the value the more "sticky" the sample. The area under the negative part of the graph is known as the adhesiveness (8.3 mJ) which is the energy required for breaking probe sample contact. These results expressed the retention time of the gel on the site of application.

3.3.2 In-vitro drug release study*:*

For the formulation L2 (20g of lawsone and 1g of carbapol) 94.20% cumulative percentage drug release up to 6 hr was observed. The *in-vitro* drug release graph is shown in Figure 5.

3.3.4 Anti-dandruff activity of formulation:

Formulation L2 batch was subjected to determination of anti-dandruff activity. Antidandruff activity of the lawsone gel was compared with the clotrimazole gel prepared by using the same base of gel. Anti-dandruff activity was determined by measuring the zone of inhibition. The formulations L2 batch shows good zone of inhibition with compared to clotrimazole gel as shown in Table 5.

4. CONCLUSION:

Considering some drawbacks of synthetic drugs, lawsone a chief chemical constituent of Henna was evaluated for its antidandruff activity and further formulated into a gel formulation. Anti-dandruff activity of lawsone was determined on the pure culture of *Malassezia Furfur*at different concentration. Lawsone showed good antidandruff activity. Lawsone gel was prepared by using carbopol 940 as a polymer in varying concentration from 0.5g-3g. After preparation of formulation the formulation were evaluated for drug content, viscosity and spreadability. In-vitro diffusion of lawsone hair gel was performed in phosphate buffer 5.0. Formulation batch L2 showed 94.20% cumulative percentage drug release up to 6 hr.Same formulations were evaluated for the zone of inhibition for their anti-dandruff activity against *Malassezia Furfur*. The formulations L2 batch shows good zone of inhibition with compared to clotrimazole gel.

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