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Research Article

Formulation and evaluation of herbal microemulsion for controlling hair loss

Nalini S. Kurup and Priyanka R. Joshi*

Department of Pharmaceutics, Prin. K. M. Kundnani College of Pharmacy, Mumbai, Maharashtra, India

ABSTRACT

Hibiscus rosa sinensis flowers and *Murraya koenigii* leaves have been reported to have the potential to control hair loss. The aim was to develop a formulation containing extracts of both herbs and test its safety and efficacy to control hair loss. Both herbs have been extracted using a suitable solvent and then incorporated into the oil phase of an oil in water microemulsion. It has been optimized for its oil phase: surfactant concentration: water phase ratio and then characterized for its particle size, zeta potential, drug release *in vitro*, *ex vivo*. Primary skin irritation and Qualitative hair growth studies have been carried out to determine the safety and efficacy of the formulation respectively. The hair growth potential has also been compared against a standard marketed formulation. The optimized microemulsion was non-irritant and had a greater efficacy to control hair loss than the standard.

Keywords: Herbal microemulsion; hair loss; *Hibiscus rosa sinensis; Murraya koenigii*

1. INTRODUCTION

In general, 50 to 100 hairs are known to be shed everyday and an increase of more than 100 constitutes a state of hair loss or alopecia. Among the various causative factors are genetic constitution, atopic state, hormonal factors, non specific immune and organ specific autoimmune reactions and possibly emotional stress, diseased state such as typhoid, malaria etc., chemotherapeutic agents and neurological factors. (Ranganathan and Shobhana, 2007, 451-55) Many substances have been investigated in attempts to treat hair loss. Topical treatment with minoxidil which is a potent vasodilator without anti androgenic properties is one of the most common approaches. (Bhalerao and Solanki, 2002, 567-73) Several herbal agents including *Hibiscus rosa sinensis* and *Murraya koenigii* are acclaimed to have hair growth promoting properties and improvement of hair quality but lack of sound scientific backing limits their use. (Jadhav et al, 2009, 454-67, Jain, 1997, 2-3, Kamboj, 2000, 35-8, Meena et al, 2010, 207-10) Topical therapy with hair oils or tonics is the most common approach for hair fall and hence microemulsions have been investigated as novel systems for the same. Microemulsions are true dispersions of one liquid within another having the following properties. (Gi et al, 1992, 665-78)

- Spontaneously forming, transparent O/W or W/O systems.
- Contain spherical particles with diameters in the range of 10 to 100nm.
- Thermodynamically stable, composed of oil, water, surfactant and co surfactant.
- Greater solubilizing capacities for hydrophilic and lipophilic drugs.

Unique microemulsions as hair care products have been prepared using a nonionic surfactant. (Paul and Moulik, 2001, 990-1001, Rosano et al, 1988, 201-9). Thus, microemulsions can be used as medicated cosmetics for reducing hair loss.

2. MATERIALS AND METHODS

2.1 Plant material

Fresh flowers of *Hibiscus rosa sinensis* and leaves of *Murraya koenigii* were purchased from the local markets near Dadar, Mumbai and authenticated from 'The Blatter Herbarium', St. Xavier's college, Mumbai.

2.2 Extraction

The herbs were shade dried for a week and then in a hot air oven (Metalab, India) at 40^0C for an hour, powdered in a grinder and sieved through #40 to acquire a fine powder. Extraction of each drug was carried out separately in a Soxhlet assembly using petroleum ether 60-80 after which they were combined in a 1:1 ratio. (Adhirajan et al, 2003, 559-63) Chemical tests were performed on both extracts for detecting the presence of their respective phytochemical constituents. (Khandelwal, 2005, 149-56) Table 2 enlists the various constituents present in both extracts.

2.3 Other materials

Camphor, Menthol, Mineral oil, Petroleum ether 60-80 (Amrut Industrial Products, Mumbai, Maharashtra, India), Isopropyl myristate (Alpha chemicals, Navi

* Corresponding Author
Email: prj_189@yahoo.com
Contact: +91-9819068649
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Mumbai, Maharashtra, India), Castor oil (Labdhi enterprises, Mumbai, Maharashtra, India), Tween 80 (Healer's nutraceuticals, Chennai, Tamilnadu, India), Ethanol (Sterling chemicals, Vadodhara, Gujarat, India), Potassium dihydrogen phosphate and Sodium hydroxide (SD fine chemicals, Mumbai, Maharashtra, India), Chloroform (Merck, Mumbai, Maharashtra, India).

2.4 Formulation of Herbal microemulsion

2.4.1 Screening of oils (Singh et al, 2010, 33-42)

Oils such as Mineral oil, Isopropyl Myristate, Camphor Menthol eutectic mixture and Castor oil were screened for their potential to solubilize the extract.

Excess of extract was dissolved in 1ml of each oil and mixed for 12hr, centrifuged at 10,000rpm for 10min (Remi, India), supernatant was separated, appropriately diluted with methanol and absorbance determined spectrophotometrically at 242nm (UV-550, Jasco, Japan). The relative solubility of extract in different oils is as shown in Table 3.

2.4.2 Construction of Pseudo-ternary phase diagrams (Attwood, 1994, 38-41, Darole et al, 2007, 122-8, Gohel and Nagori, 2010, 189-96, Rhee et al, 2001, 161-70)

Surfactant/Co-surfactant combinations (S/C=3:1, 3:2 ratios) and Eutectic mixture (camphor menthol in equal parts) were taken in ratios of 1:9 to 9:1 in 9 separate beakers. These mixtures were titrated with water under gentle agitation (using a magnetic stirrer) until the first turbidity appeared. The quantities of three phases were substituted in the CHEMIX school software (version 3.51) and pseudo-ternary diagrams were constructed as denoted in Fig.1. Since both ratios yielded the same area in O/W microemulsion zone, the ratio with higher solubility for the extract was selected.

2.4.3 Preparation of Microemulsion batches

Extract was dissolved into the oil phase and S/C mixture was added. The mixture was stirred magnetically (Magnetic stirrer, Remi, India) to achieve homogeneity. Water was added drop wise to obtain a clear, transparent microemulsion. The various batches formulated have been listed in Table 4.

2.5 Characterization of Herbal Microemulsion

2.5.1 Determination of particle size and polydispersity index

Principle of photon correlation spectroscopy (PCS) was utilized to assess mean particle size (z Avg.) and polydispersity index of the formulated batches using the Malvern particle size analyzer (Zetasizer, UK) instrument. The results are listed in Table 5 and Fig.2.

2.5.2 Determination of Zeta potential

All the measurements were performed in triplicate using Malvern Zetasizer instrument. The results are listed in Table 6 and Fig.3.

2.5.3 *In vitro* drug release study (Abd-Allah et al, 2010, 257-66, Kogan and Garti, 2006, 369-85)

The batches that had lower polydispersity index and higher zeta potential were selected for evaluating their drug release (A1 and A3). *In vitro* drug release study of these batches was performed in a Keshary Chien diffusion cell using dialysis membrane soaked in Millipore water for 12h.

The receiver chamber was filled with phosphate buffer pH 7.4 with 5% Tween 80 and temperature of the medium was thermostatically controlled at 37 ± 1^0C. Accurately measured 1ml of microemulsion was applied to the donor compartment. 1ml sample was withdrawn over a period of 24h and analyzed using spectrophotometer at 242nm, using phosphate buffer of pH 7.4 as blank. The results are denoted in Fig.4.

2.5.4 *Ex vivo* drug release study (Singh et al, 2010, 33-42, Subramanian, 2004, 335-41)

The batch that yielded a faster drug release *in vitro* was selected for evaluation of drug release *ex vivo.*

Ex vivo drug release study of batch A3 was performed in a Keshary Chien diffusion cell, using rat abdominal skin instead of the dialysis membrane used for *in vitro* studies. The skin was mounted between the donor and receiver compartments where the stratum corneum side faced the donor compartment and the dermal side faced the receiver compartment. The results are denoted in Fig.5.

2.6 In vivo studies of Herbal microemulsion

2.6.1 Primary skin irritation test (Adhirajan et al, 2001, 559-63, Chen et al, 2007, 78-84, Dhamankar et al, 2009, 1449-57, Pansang et al, 2010, 355-8)

Three healthy female New Zealand white rabbits weighing 2.5 to 3.0kg were selected for the study. 24h prior to the test, the hair from the back of each rabbit was shaved to accommodate test sites of $4.0cm^2$.

Measured quantity of microemulsion was applied over the test sites and covered with surgical gauze and observed for erythema, edema and eschar formation for 72h after application. Image of rabbit skin after 72h is denoted in Fig.6.

2.6.2 Qualitative Hair Growth studies

The rats were divided into three groups of six rats each. Each group had equal number of males and females. The groups were labeled as per Table 1.

Table 1: Rat groups for Qualitative hair growth studies

Group	Treatment given
I. Control	No treatment
II. Standard	5% Minoxidil topical solution
III. Test	4% Herbal Microemulsion

A $4cm^2$ area of hair from the dorsal portion of all the rats was shaved off and wiped with surgical spirit. One

ml of the microemulsion and standard was applied to the denuded area of the respective groups once a day. This treatment was continued for 30 days, during which period, hair growth initiation and completion time was noted. (Adhirajan et al, 2001, 559-63, Purwal et al, 2008, 34-8, Roy et al, 2006, 951-6, Yoon et al, 2010, 1350-4) Results are denoted in Table 7 and Fig.7.

Hair was plucked randomly from the test area of each rat from each group on the 7th, 14th, 21st and 28th day of the treatment. The length of 10 hairs per animal was measured and the average length was determined. (Adhirajan et al, 2003, 235-9) Results have been listed in Table 8.

3. RESULTS AND DISCUSSION

3.1 Determination of phytochemical constituents of extracts

Based on the phytochemical evaluation, the constituents that were detected in the petroleum ether extracts of *Hibiscus rosa-sinensis* and *Murraya koenigii* have been summarized in Table 2.

Table 2: Phytochemical constituents of herbal extracts

Extract	Constituent present
Hibiscus rosa-sinensis	Steroids
Murraya koenigii	Steroids, Alkaloids, Essential oils

3.2 Formulation of the Herbal microemulsion

3.2.1 Screening of oils

Table 3: Relative solubility of extract in various oils

Oils	Relative Solubility of extract in oils[b] (mg/ml)
Camphor Menthol eutectic mix	106 ± 1.00
Mineral oil	7.14 ± 1.30
Isopropyl Myristate	6.36 ± 0.40
Castor oil	2.05 ± 0.50

[b] Values expressed as ±S.D. (n=3)

Since, the extract was found to have highest solubility in camphor-menthol eutectic mixture, it was selected as the oil phase for the formulation.

3.2.2 Construction of pseudo-ternary phase diagrams

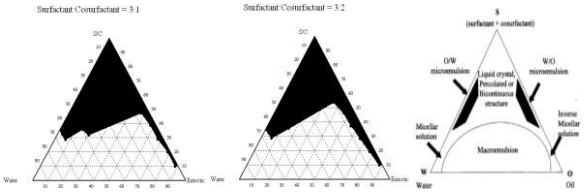


Figure 1: Pseudo ternary phase diagrams for two different ratios of S/C and hypothetical phase regions for microemulsion systems

Both ratios resulted in an equal area of existing microemulsion and hence, the one that solubilized more amount of extract was selected for the optimized batch.

The translucent microemulsion region is presented in phase diagrams. The rest of the region represents the turbid and conventional emulsion based on visual observation. The area of microemulsion region changed slightly in size with the increasing ratio of surfactant to cosurfactant.

Ethanol distributed itself between aqueous and oily phase, thereby altering the chemical composition and hence relative hydro/ lipophilicity of the system. At the optimum S/C value, the cosurfactant was inserted into the cavities between the surfactant molecules exactly, and the formed microemulsion had the maximum solubilisation capacity. The liquid crystal or bicontinuous structure area was greater in ratio 3:1 because ethanol is a polar solvent with the tendency to highly incorporate into water, and the relatively lower ethanol content in the microemulsion systems decreased the hydrophilicity of the surfactant mix. (Kumar et al, 2010, 57-74, Singh et al, 2010, 33-42, Kogan and Garti, 2006, 369-85)

3.2.3 Composition of microemulsion batches

Table 4: Various batches of microemulsion formulated

Batch no.	Camphor+ Menthol (1:1) (%w/w)	Tween80+ Ethanol (3:2) (%w/w)	Distilled water (%w/w)	Herbal extract in oil (g)
A1	10	40	50	4
A2	10	37	53	4
A3	10	33	57	4
A4	10	30	60	4

3.3 Characterization of Herbal microemulsion

3.3.1 Determination of particle size and polydispersity index

In the present study, the average particle size of all formulations was in the range of 59.27 to 132.7 nm. The results of average particle size and polydispersity index for all batches are listed in Table 5. A higher concentration of surfactant resulted in finer droplet size. However, it also resulted in corresponding increase in viscosity of the formulations. The mean particle size and width of distribution of Batch A3 is shown in Fig.2. Polydispersity index of all batches was in the range of 0.176 to 0.353, thus the herbal microemulsions showed a narrow distribution width and considerably small particle size.

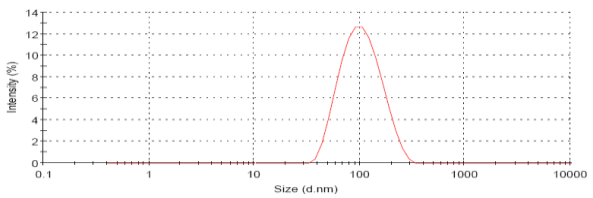


Figure 2: PCS image of particle size distribution of optimized batch A3

Table 5: Results of Average Particle size and Polydispersity Index

Batch No.	Avg. Particle Size (nm)[b]		Polydispersity Index[b]	
	Mean	±S.D.	Mean	±S.D.
A1	59.27	±3.8	0.182	±0.008
A2	84.24	±10.6	0.353	±0.013
A3	**107.7**	**±2.6**	**0.176**	**±0.004**
A4	132.7	±6.9	0.320	±0.016

[b] Values expressed as Mean±S.D., n= 3

3.3.2 Determination of Zeta potential (Hadkar, 2003, 99-101, Martin et al, 1991, 482, 485, 486, Sharma and Suresh, 2010, 66-73)

The analysis of zeta potential is a useful tool to estimate the stability of dispersed systems. The experimental measurements are indicated in Table 6 which shows that only Batch A3 had the highest zeta potential value, indicating the best stability while Batch A1 also has a high value. Hence, particle aggregation would not be likely to occur owing to electrostatic repulsion between the particles. Fig.3 indicates the zeta potential measurements of optimized batch A3. All formulations exhibited negative values.

Table 6: Zeta potential measurements of various formulations

Batch No.	Zeta potential	
	Mean	**±S.D. (n=3)**
A1	-28.3	±3.8
A2	-18.9	±2.9
A3	**-32.0**	**±1.9**
A4	-11.4	±2.7

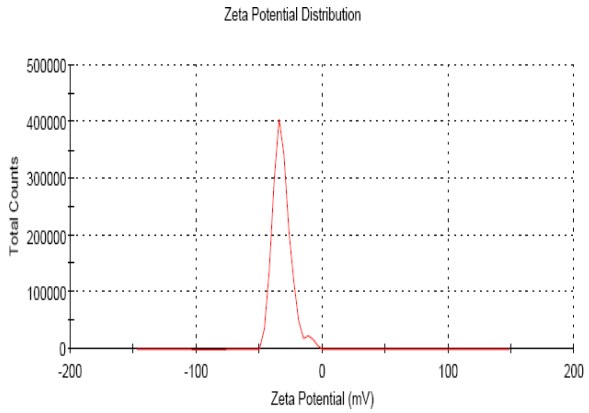


Figure 3: Zeta Potential of Batch A3

3.3.3 *In vitro* drug release study

The cumulative percentage release from the two batches A1 and A3 was investigated for a period of 24h (Fig.4). The release was found to be slower from Batch A1 ie 62.0% while it was 81.31% for A3. This may be related to the surfactant concentration. A1 had a smaller particle size owing to higher surfactant concentration and hence a higher viscosity and therefore exhibited a slower release.

3.3.4 *Ex vivo* drug release study

The *ex vivo* study of Herbal microemulsion A3 is depicted in Fig.5. Cumulative amount of drug release after 24h was found to be 85.64%.

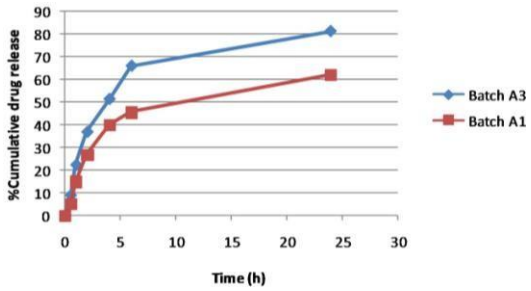


Figure 4: In vitro drug release from Batches A1 and A3

The exact mechanism of action of the extract is unknown; however, a hypothesis has been formulated. It states that the extract improves the blood supply to the follicles and hence converts some of the hair follicles from the telogen to anagen phase. This results in an increase in the anagen to telogen ratio and hence the converted follicles are not shed for a longer time, thus, effectively reducing hair loss. However, the extract cannot produce hair growth in an area that is already bald or devoid of hair follicles. (Sabarwal et al, 2009, 165-70)

As a result, a faster release can prove beneficial and therefore, Batch A3 has been deemed to be the optimized one.

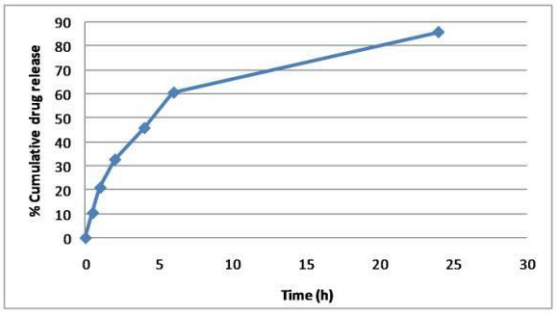


Figure 5: Ex vivo skin release of microemulsion

3.4 In vivo studies of Herbal microemulsion

3.4.1 Primary skin irritation test

No erythema and edema was noted after 72h and hence the formulation was classified as non irritant.

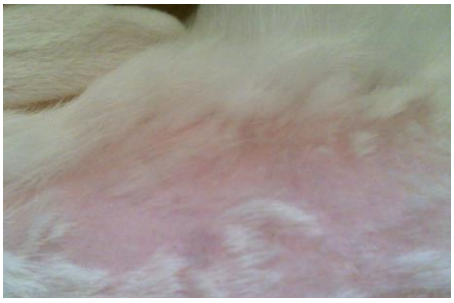


Figure 6: Image of rabbit skin upon treatment with microemulsion

3.4.2 Qualitative Hair growth study

Throughout the 30 day study period, every animal of each group was observed closely to determine the hair length initiation and completion time. This was achieved using a magnifying lens that enabled observation of minute changes in the hair growth pattern. The point at which a tiny prickle of hair growth was observed was noted as the initiation time.

The hair growth initiated and completed in the following order: Microemulsion > Standard > Control

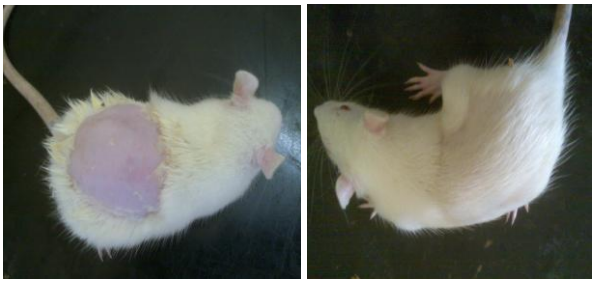
Table 7: Hair growth initiation and completion time of different groups

Group	Initiation time (days)	Completion time (days)
Control	10	25
Standard	7	23
Microemulsion	4	19

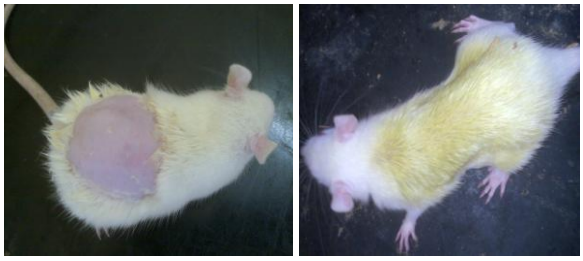
The microemulsion treated groups produced a greater effect on the length of hair when compared to other groups. This may be due to the premature switching of follicles from the telogen to anagen phase of hair growth cycle.

Moreover, in all the groups except the microemulsion treated group, the hair looked sparse. This explains the presence of greater number of hair follicles in the anagen phase of the hair growth cycle in microemulsion treated groups. A study has been carried out by Adhirajan et al. (2003), which proved that the hair follicles periodically transformed from telogen to anagen phase in groups treated by hibiscus flower extract by proliferation of cells in the telogen phase follicles resulting in their conversion to the anagen phase. (Paus, 2006, 101-10, Philpot et al, 1992, 600-7).

A mechanism of conversion of short vellus hairs to long terminal hairs and an enlargement of the follicular size with prolongation of anagen phase by enhancing the rate of cell proliferation has been proven for minoxidil. Also the induction of proliferation of epithelial cells near the base of the hair follicle and vasodilation of scalp blood vessels has been proven. (Uno and Kurata, 1993, 143-7) Hence, it was thought that the formulations evaluated in this work could be acting in a similar manner.



Control group



Microemulsion group

Figure 7: Hair growth at Days 0 and 28 of control and test groups

Average hair length of each group at various time intervals of a 30 day study period has been listed in Table 8. Statistical significance for the study was determined using InStat version 3 software.

The values of the control group were compared to the corresponding values of all other groups using one way ANOVA test followed by Tukey Kramer post test

Table 8: Average hair length of different groups at various time intervals

	Average hair length (mm)			
Group	Day 7[b]	Day 14[b]	Day 21[b]	Day 28[b]
Control	3.00± 0.267	6.69± 0.210	11.81± 0.266	15.06± 0.199
Standard	8.13± 0.246***	12.25± 0.211***	15.00± 0.164***	16.94± 0.148***
Microemulsion	11.38± 0.220***	15.38± 0.206***	17.25± 0.189***	21.69± 0.188***

[b] Values expressed as ±S.E.M., n=60 hairs

*** $p < 0.001$ when control group was compared with all other groups

CONCLUSION

A Herbal microemulsion was successfully formulated by incorporating petroleum ether extracts of *Hibiscus rosa sinensis* and *Murraya koenigii* in a 1:1 ratio. Drug release from microemulsion was found to be 81.31% *in vitro* and 85.64% *ex vivo*. *In vivo* studies proved that it was non irritant and more effective than standard for controlling hair loss. Thus, a safe and efficacious herbal microemulsion was developed.

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