



Formulation and Evaluation of Anti-fungal cream containing Ethanolic Extract of *Leonotis nepetaefolia* (L) R.Br.

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ABSTRACT

Fungal infection is now-a-days affecting almost every human being. In traditional system of medicine Indian population depends on this indigenous system for the treatment of fungal infection. Leonotis nepetaefolia (L) R.Br. belongs to family Lamiaceae. The flowers of the plant are used medicinally for the treatment of several skin and fungal infections. In the present paper attempt was made to formulate and evaluate the herbal cream containing ethanolic flower extract of the plant. Different batches viz., AFHC1 to AFHC8 were prepared using different ratio of ingredients and were evaluated. The results of evaluation parameters revealed that AFHC5 have best results when compared with other formulation codes.

Keywords: *Leonotis nepetaefolia (L) R.Br., Cream, Anti-fungal*

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INTRODUCTION

Leonotis nepetaefolia (L) R.Br. commonly known barchibuti as is a less known medicinal plant, belongs to the family Lamiaceae. It is found almost along the hotter parts of India. Traditionally, the all parts of the plant especially roots, leaf and flowers are used for rheumatic problems and also serves as a tonic. Flower heads are used against scalds, burns, ringworm and some skin diseases [1-3].

In the last few years there has been an exponential growth in the field of herbal medicine and the formulation made by them in alone or in combination using extract. In Indian systems of medicine most practitioners formulate and dispense their own recipes using crude drug as such or using the extract of the plant [4]. So, far no any systematic study was carried out in formulation the dosage form using flowers of selected plant, therefore, the present work was undertaken to formulate and evaluate anti-fungal cream containing ethanolic extract.

MATERIAL AND METHODS

Collection of herbs and their authentication

The flowers of *Leonotis nepetaefolia* (L) R.Br. (LNF) were collected in the months of July-September 2020 from the various local sites of Malwa region of Madhya Pradesh and identified & authenticated by Dr. S. N. Dwivedi, Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, (M.P.) and was deposited in our Laboratory. Voucher specimen No. J/Bot/2020-LNF392 was allotted.

Extraction of selected herbs

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered plant material (250gms) were loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and was used accordingly [5, 6].

Plant extracts

The ethanolic extracts of dried plant material of *Leonotis nepetaefolia* (L) R.Br. (LNF) (Flowers) were taken for formulation.

Formulation of herbal cream [7, 8]

The various steps involved in formulation of herbal cream were mentioned as described below [5, 6]:

Preparation of oil phase

Stearic acid, cetyl alcohol, almond oil in desired quantity were taken in porcelain dish and was melted at 70°C.

Preparation of aqueous phase

Alcoholic extracts of dried plant material of *Leonotis nepetaefolia* (L) R.Br. (LNF) (Flowers), glycerol, methyl paraben, triethanolamine and water were taken in another porcelain dish and were heated at 70°C.

Addition of aqueous phase to oil phase

The aqueous phase was added to the oil phase with continuous stirring at room temperature. Perfume was added at last and the formulation was transferred in a suitable container.

Table 1: Formulation of herbal cream containing ethanolic extract of *Leonotis nepetaefolia* (L) R.Br. (LNF) (Flowers)

Ingredients	Formulation Code (EALNF)							
	HC1	HC2	HC3	HC4	HC5	HC6	HC7	HC8
EELNF	0.5	0.75	1.0	1.5	0.5	0.75	1.0	1.5
Stearic acid	5	5	5	5	10	10	10	10
Cetyl alcohol	10	10	10	10	5	5	5	5
Almond oil	5	5	5	5	5	5	5	5
Glycerol	3	3	3	3	3	3	3	3
Methyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Triethanolamine	qs	qs	qs	qs	qs	qs	qs	qs
Water (100 ml)	qs	qs	qs	qs	qs	qs	qs	qs
Total weight	100	100	100	100	100	100	100	100

Note: All values are taken in gm

Evaluation parameters of herbal cream

The prepared formulations were evaluated for the following parameters [7, 8]:

Physical evaluation

The physical evaluation of the herbal cream was done by evaluating clarity and transparency which was determined visually. The samples were observed in light at white background.

Determination of pH

The pH meter was calibrated first and zero reading was recorded. The samples were taken in the beaker and the readings were taken from calibrated electrode. The procedure was repeated and three average reading was recorded.

Determination of Viscosity

The viscosity of the herbal cream was determined by Brookfield viscometer using spindle no 01 at 20 rpm at temperature 4 °C and 37°C. About 15ml of the was taken in beaker and spindle was immersed in the formulation. The reading was recorded at initial and after rotation at different temperature. The reading was recorded thrice.

Determination of Homogeneity

All the prepared herbal cream was tested for homogeneity by visual inspection and was evaluated for presence of any aggregates present in the formulation.

Determination of Spreadability

The spreadability was determined for all the prepared herbal cream. The formulations were placed on the glass slide and the empty glass slide was placed on the top of gel containing slide. The formulation was placed in such a way that it was placed between two slides. The occupied distance of the slides was observed to be of 7.5 cm. The herbal cream was placed between slide and pressed form thin uniform layer. The weight kept on the herbal cream was removed. The excess herbal cream observed in the slides was removed. The two slides were fixed and on the upper glass slide the 20 ±0.5 g of the weight was tied. Due to weight the both the slides were separated which was recorded as time to complete the separation distance of 7.5 cm. The three readings were recorded and mean time was taken. The spreadability was calculated as

$$S = m \times l / t$$

l is the length of slide (7.5 cm), m is the weight which is tied to slides and t is the time taken in second.

Determination of type of smear

The prepared herbal cream was applied on the skin surface and after the application the type of film or smear formed on the skin was recorded.

Determination of Emolliency

The prepared herbal cream was checked for emolliency, slipperiness and amount of residue left after the application of cream.

Determination of type of Emulsion

Dilution test

The prepared herbal cream was diluted with oil or water depending upon the type of emulsion whether o/w or w/o the results obtained were noted down.

Dye solubility test

The prepared herbal cream was mixed with a water soluble dye i.e., amaranth and was observed under the microscope. The results obtained were interpreted.

Determination of Drug content

The content of the herbal cream was estimated using UV-Visible spectrophotometer. Near about 1g of the formulation was taken in 50 ml of volumetric flask. The solution was made up to mark with methanol. The solution was shaken and filtered through Whatman filter paper. The 0.1ml of the filtrate was further diluted to 10ml with solvent and estimated at suitable wavelength.

In vitro drug release

The semi permeable dialysis membrane bag (7cm long) was prepared and the herbal cream was placed in the membrane. The dialysis bag was then suspended in 50ml of ethanol: water (1:1) at temperature $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ in water bath. About 1ml of sample was withdrawn from the membrane at predetermined interval and the fresh equal volume was replaced simultaneously. The samples were withdrawn till one week and were diluted and analyzed by UV Visible spectrophotometer at suitable λ_{max} . The experiment was repeated thrice and the cumulative amount of drug release was calculated from the reading.

RESULTS AND DISCUSSION

The investigation of the efficiency of plant extract and their formulations in induced systemic and local infection model is of quite interesting. Several researchers have evaluated the effects of plant extracts along with their formulations in systemic infections and in the treatment of fungal infection. It was also noted that now-a-days there are several herbal formulations are in the market used for the fungal infection and they having very less or no adverse/side effects. The present work was undertaken to develop and evaluate herbal cream containing ethanolic extract of *Leonotis nepetaefolia* (L) R.Br. (LNF) (Flowers). The selected extract viz., EELNF along with various excipients selected were mixed according to the formula mentioned and various evaluation parameters were carried out to validate the efficacy of the prepared formulation. The formulated herbal cream containing was evaluated as per standard protocols. The detail results are mentioned in table 2 & 3.

The drug content was found maximum in AFHC5 i.e., 99.10% (Table3). Therefore, that formulation is taken in consideration for determination of drug release. The results of drug release profile indicate that the formulation AFHC5 has maximum release of 97.48 % at 8 hr. (Table 4)

Table 2: Evaluation parameters of herbal cream containing ethanolic extract of *Leonotis nepetaefolia* (L) R.Br.

FC	Appearance	pH	Viscosity	Homogeneity	Spreadability	Smear	Emolliency	Emulsion
AFHC1	Off White & Clear	7.1	26032	H	63.24	NG	NRL	o/w
AFHC2	Off White & Clear	6.9	26012	H	60.32	NG	NRL	o/w
AFHC3	Off White & Clear	7.1	26045	H	61.27	NG	NRL	o/w
AFHC4	Off White & Clear	7.0	26016	H	65.64	NG	NRL	o/w
AFHC5	Off White & Clear	7.0	26018	H	60.14	NG	NRL	o/w
AFHC6	Off White & Clear	7.1	26016	H	62.37	NG	NRL	o/w
AFHC7	Off White & Clear	7.0	26024	H	61.27	NG	NRL	o/w
AFHC8	Off White & Clear	6.8	226010	H	60.34	NG	NRL	o/w

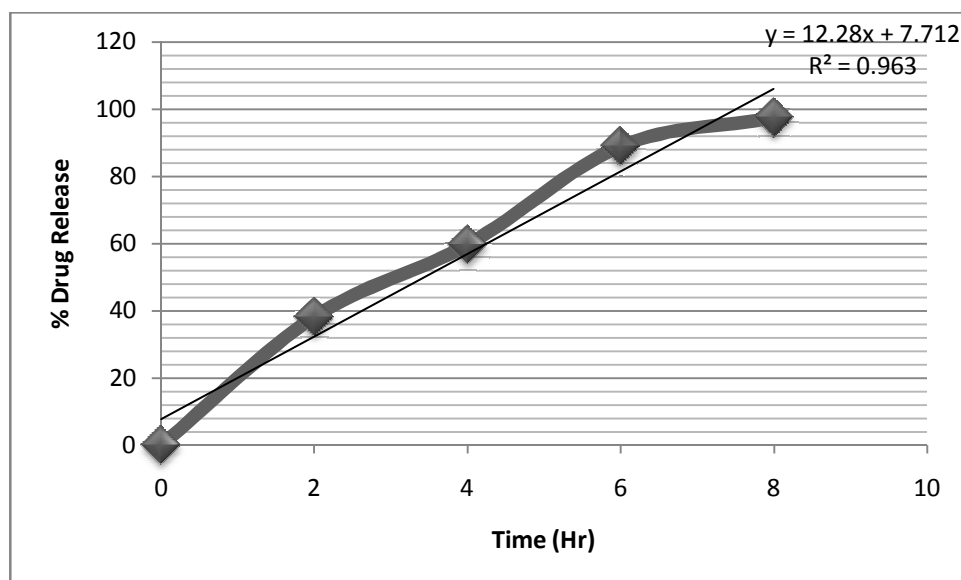
Note: H=Homogeneous, NH=Non homogeneous,, G=Greasy, NG= Non-greasy, NRL=No residue left, LR=Residue left

Table 3: Drug content of herbal cream containing ethanolic extract of *Leonotis nepetaefolia* (L) R.Br

FC	Drug content (%)
AFHC1	94.87
AFHC2	95.21
AFHC3	96.24
AFHC4	97.31
AFHC5	99.10
AFHC6	97.24
AFHC7	95.20
AFHC8	94.89

Table 4: % Drug release of optimized herbal cream (AFHC 5) containing ethanolic extract of *Leonotis nepetaefolia* (L) R.Br

Time (Hr)	% Drug Release
0	0
2	38.21
4	59.62
6	88.95
8	97.48

**Graph 1: % Drug release of AFHC 5****CONCLUSION**

From the results obtained it was concluded that the ethanolic extract of *Leonotis nepetaefolia* (L) R.Br (Flowers) have effective results when formulated in the form of cream. The formulation code AFHC5 has promising and effective drug content and release. Hence, it was concluded from the present investigation that the selected herbs will have a prominent effect in the treatment of fungal infection, though the pharmacological screening and clinical approaches need to establish for the formulation of safe and effective herbal drugs.

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