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Research Article

Formulation and Evaluation of Herbal Ethosomal Suspension

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Abstract

Cosmaceuticals has been used to describe the products that yield benefits traditionally, and active constituents thought to be cosmetic in nature, such as moisturization, as well as product that make marketing claims approaching those of drug products, such as reducing wrinkles, regenerates skin, firms, heal and penetrates into skin. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. In the undertaken study an attempt has been made to establish that herbal ethosomal suspension containing *Azadirachta indica* extract has promising anti-aging action. The entrapment efficiency of formulation containing ethanolic extract FE8 was found to be highest (75.91%) while FE3 formulation showed least entrapment efficiency (66.66 %). However it has been observed the formulation containing phospholipid (3 gm) with ethanol has maximum entrapment efficiency.

Keyword : Cosmaceuticals, Natural remedies. *Azadirachta indica,* Ethosomal suspension

Introduction : Cosmaceuticals has been used to describe the products that yield benefits traditionally, and active constituents thought to be cosmetic in nature, such as moisturization, as well as product that make marketing claims approaching those of drug products, such as reducing wrinkles, regenerates skin, firms, heal and penetrates into skin. Consumers seek "anti-wrinkle" cosmetic products that treat or delay the visible signs of actual aging and weathered skin, such as wrinkles, lines, sagging, and hyper pigmentation and age spots. The present consumers are highly specific about choice of product; consumers prefer mainly products free of synthetic active constituents, synthetic preservatives and base free of animal derivatives. Unnatural, chemically-synthesized products may be perceived as being environmentally or personally unsafe .However, delivering "natural" sources, such as plants or herbs, through conventional formulation is not enough for treatment of fine lines, indeed lines and furrows appears at epidermal surface but their main cause of appearance is at deeper layer. [1,2]

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In contrast, natural products are perceived as pure, mild, and superior to chemically synthesized products. Natural based products extracted from plants or herbs are believed to contain antioxidant/free-radical scavenging agents that can neutralize the effects of free-radical damage [3,4]. Additionally, they contain agents that stimulate the synthesis and restoration of damaged connective tissue structures in the dermis and barrier function in the epidermis

Ethosomes are soft, malleable, tiny bubble like lipid vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water. These "soft vesicles" represents smart vesicular carrier for enhanced delivery to/through skin. All components of the **Ethosomal systems** are considered as being safe for pharmaceutical and cosmetic use. Ethosomal systems were found to be significantly superior at delivering drugs through the skin in terms of both quantity and depth when compared to liposomes and to many commercial transdermal and dermal delivery systems**.** Ethosomes are sophisticated vesicular delivery carriers that are capable of delivering various chemical applications. [3,-5]

Materials and Methods

Preparation of extracts:

The dried powder of plant leave was extracted with Ethanol using Soxhlet apparatus.

About 250 gm of dried powder leave of plant was subjected to soxhlation. It was first defatted with petroleum ether then exhaustively extracted with solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract[6,7]

Preparation of Ethosomes suspension:

Ethosomes were prepared by solvent dispersion method: Soya phospotidylcholine up to (2-3%) ,2.5 gm of extract taken and dissolved in (30-40%) of 90% ethanol by use of magnetic stirrer (Remi Motors Mumbai), to this solution fine stream of distilled water was added with help of syringe, then whole system was stirred for 30 minutes at 700 rpm. Some formulation with treatment of SLS and Tween 80 were prepared for increasing solubility of extract.[8-10] (Table 1)

Evaluation of Ethosome suspension[8-13]:

Image analysis of ethosomes by optical microscope

Visualization done by image analysis compound microscope .The compound microscope is attached with the digital camera: Nikol, coolpix, L20, through which image analysis was done, photographs were captured.

Vesicular shape and surface morphology

Transmission Electron Microscope (TEM) was used as a visualizing aid for ethosomal vesicles. Samples were dried on carbon-coated grid and negatively stained with aqueous solution of phosphotungstic acid. After drying the specimen was viewed under the microscope.

Determination of Entrapment efficiency of ethosomes suspension:

Aliquots of ethosomal suspension (10 ml) were subjected to centrifugation using cooling ultracentrifuge (Remi) at 12000 rpm for 90 minutes. The clear supernatant was siphoned off carefully and the absorbance was recorded at λmax 415 nm using UV/Vis spectrophotometer (Shimadzu UV 1700). The percent entrapment was calculated using the formula.

% EE = [Qt-Qs/Qt] X100

Where, EE is the entrapment efficiency, Qt is amount of extract added, Qs is amount detected in the supernatant.

Result and Discussion –

EXTRACTION

The dried powder of plant was extracted with ethanolic solvents. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract. The percentage yields of various extract was presented in Table 2.

S/No.	Type of Extract	%Yield (w/w)	Color of Extract
1.	Ethanolic extract	13.7123	Dark Green

Evaluation of Ethosome suspension

Image analysis of ethosomes by optical microscope

For the initial vesicle characterization of ethosome suspension were examined by compound microscope. The result revealed that formulation without added SLS and Tween 80 shown aggregation process among the structure . Formulation in which SLS and Tween 80 added shown spherical shaped vesicles like structure without aggregation process.(Fig 1) Hence, Formulation containing SLS and Tween 80 in ehanolic extract (FE3, FE4,FE7,FE8) were considered for further study and remaining formulation batches were discarded.

Vesicular shape and surface morphology by TEM

The vesicular shape and surface morphology of ethosomes of formulation FE8 examined by Transmission Electron Microscope (TEM). The TEM image showed that ethosomes were spherical shaped. .

Determination of Entrapment efficiency of ethosomes suspension:

The entrapment efficiency of various ethosomes formulations are presented in Table.. The entrapment efficiency of formulation containing ethanolic extract FE8 was found to be highest (75.91%) while FE3 formulation showed least entrapment efficiency (66.66 %). However it has been observed the formulation containing phospholipid (3 gm) with ethanol has maximum entrapment efficiency.

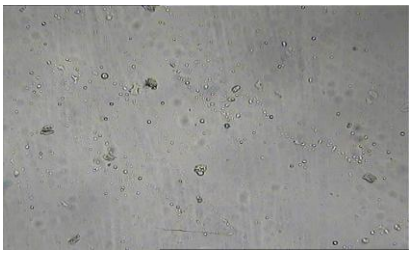

Figure 1: Microphotographs of Ethosome suspensions containing *Azadirachta indica* plant extract

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Table 1: Preparation of Ethosomes suspension containing Ethanolic extract

Ingredient	***FE1***	***FE2***	***FE3***	***FE4***	***FE5***	***FE6***	***FE7***	***FE8***
Azadirachta indica Ethanolic Extract (% w/w)	*2.5*	*2.5*	*2.5*	*2.5*	*2.5*	*2.5*	*2.5*	*2.5*
Phospholipid	*2*	*2*	*2*	*2*	*3*	*3*	*3*	*3*
Ethanol	*30*	*40*	*30*	*40*	*30*	*40*	*30*	*40*
Tween 80	*-*	*-*	*2 ml*	*2ml*	*-*	*-*	*2 ml*	*2ml*
SLS	*-*	*-*	*500mg*	*500mg*	*-*	*-*	*500mg*	*500mg*
Distilled Water	*q.s. to 100ml*	*q.s. to 100ml*	*q.s. to 100ml*	*q.s. to 100ml*	*q.s. to 100ml*	*q.s. to 100ml*	*q.s. to 100ml*	*q.s. to 100ml*

Each formulation contains distilled water up to 100 ml

Table 3. Entrapment efficiency of ethosomes suspension

S/No.	**Ethosomes**	**Qt**	**Qs**	**%EE**
1.	FE3	2.5	0.8326	66.66
2.	FE4	2.5	0.7916	68.33
3.	FE7	2.5	0.6921	72.31
4.	FE8	2.5	0.6021	75.91

EE =entrapment efficiency, Qt = amount of extract added, Qs = amount detected in the supernatant.