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Formulation and Evaluation of Ethosome containing Fruit Extract of Phyllanthus emblica Linn

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Abstract
The present paper deals with the formulation and evaluation of ethosome formulation containing of Hydroalcohalic extract of *Phyllanthus emblica Linn* fruit, Tee Tree oil and Avocado oil. Ethosomes were prepared by solvent dispersion method: Soya phospotidylcholine up to (2-3%)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. entrapment efficiency of formulation containing *Phyllanthus emblica Linn* hydroalcohalic extract FA8 was found to be highest (73.32%) while FA3 formulation showed least entrapment efficiency (60.27 %). It has been observed the formulation containing phospholipid (3 gm) with 40mL ethanol has maximum entrapment efficiency.

Keywords: Ethosome, hydroalcohalic extract, ***Phyllanthus emblica Linn*** ,Tee Tree oil and Avocado oil. ,

Introduction

The use of medicinal plants as raw materials in the production of new drugs is ever increasing because of their potentials in combating the problem of drug resistance in micro-organisms. Demand for medicinal plants is increasing in both developing and developed countries. Research on medicinal plants is one of the leading areas of research globally[1-2]. However, there is a need to pay closer attention to the issue of bioactivity-safety evaluation of medicinal plants.

E. officinalis Linn.(Syn. Phyllanthus emblica Linn.), the Indian gooseberry, or aamla', is a deciduous tree. Emblica is used to promote the growth of hair in traditional medicine. Embelica is reported to improve the iron metabolism; Iron is involved in the oxygenation of our body's red blood cells. It is essential for normal hair growth and for the maintenance of healthy hair. Iron deficiency leads to hair loss because of oxygen deficiency. Embelica extracts stimulate proliferation of dermal papilla cell in a concentration dependent manner, suggesting their role in hair growth promotion .[1-7]

Avocado fruit are well-known, with millennia of consumption in the Americas and an increasing popularity in the rest of the world. roughout history, avocado oil was renowned for its healing and regenerating properties. Early writings from the sixteenth century reported the use of the oil obtained from the seed to treat rashes and scars (Argueta-Villamar et al., 1994). In skin care, the two major advantages of avocado oil are its marked softening and soothing natureand its notable absorption.

For example, compared with almond, corn, olive, and soybean oils, avocado oil had the highest skin-penetration rat

Material & Methods

Collection of plant material

The seeds of *Trigonella foenum greacum, Phyllanthus emblica Linn* fruit and *Allium cepa L* were obtained from local market while Avocado oil , Tee Tree oil were purchase from market .

Preparation of extracts

About 250-250 gm of dried powder of freshly cutted small pieces *Phyllanthus emblica Linn* fruit were subjected to soxhlation separately. It was first defatted with petroleum ether then exhaustively extracted with ethanol 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. [7-8]

Preparation of Ethosomes suspension :

Ethosomes were prepared by solvent dispersion method: Soya phospotidylcholine up to (2-3%) ,10 gm of *Phyllanthus emblica Linn* fruit extract, 7.5 ml Avocado oil and 5 ml Tee Tree oil 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. (Table 1) [9-16]

Table 1: Preparation of Ethosomes suspension containing Ethanolic extract of *Phyllanthus emblica Linn* fruit

Ingredient	FA1	FA2	FA3	FA4	FA5	FA6	FA7	FA8
Phyllanthus emblica Linn fruit (% w/w)	10	10	10	10	10	10	10	10
Tee Tree oil	5	5	5	5	5	5	5	5
Avocado oil (% w/v)	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
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.

Evaluation of Ethosome suspension: [9-16]

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 255 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.

RESULTS AND CONCLUSION

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.Hence, Formulation containing SLS and Tween 80 in ehanolic extract **of *Phyllanthus emblica Linn* fruit** (FA3, FA4, FA7, FA8) were considered for further study and remaining formulation batches were discarded.For the initial vesicle characterization of ethosome suspension were examined by compound microscope. The result revealed that all formulation shown spherical shaped vesicles like structure without aggregation process.

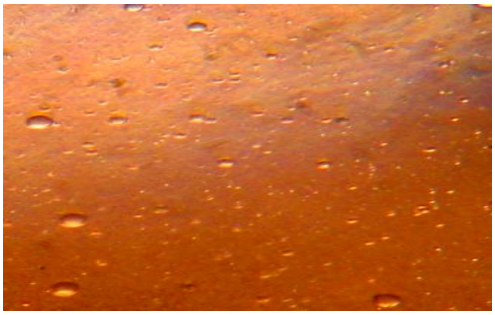
Determination of Entrapment efficiency of ethosomes suspension:

The entrapment efficiency of various ethosomes formulations are presented in Table.3.9 The entrapment efficiency of formulation containing *Phyllanthus emblica Linn* ethanolic extract FA8 was found to be highest (73.32%) while FA3 formulation showed least entrapment efficiency (60.27 %). It has been observed the formulation containing phospholipid (3 gm) with 40mL ethanol has maximum entrapment efficiency.

Table 2: Entrapment efficiency of ethosomes suspension

S/No.	Ethosomes	Qt	Qs	%EE
1.	FA3	2.5	1.0181	60.27
2.	FA4	2.5	0.8797	65.81
3.	FA7	2.5	0.7389	71.44
4.	FA8	2.5	0.6919	73.32

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



Ethosome suspension, FA8

Figure 1: Microphotographs of Ethosome suspensions containing *Phyllanthus emblica Linn* fruit extract

Vesicular shape and surface morphology by TEM

The vesicular shape and surface morphology of ethosomes formulation **(**FA8) examined by Transmission Electron Microscope (TEM). The TEM image showed that ethosomes were spherical shaped. (fig.2)

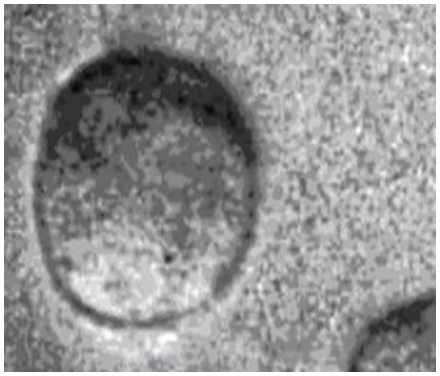


Figure 2: Transmission electron microphotograph Visualization of ethosomes FA8.

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