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DEVELOPMENT OF FORMULATION AND EVALUATION OF PHYTOSOMAL GEL OF NEEM

Dr. Vasuki K[1]*, Dr. Manivannan R[2], Abinaya R, Pavithra M, Tamilselvan S, Vimalya S.

[1]Professor, Department of Pharmacognosy, Excel College of Pharmacy, Komarapalayam, Namakkal Dt, Tamil Nadu - 638 183, India.
[2]Principal, Excel College of Pharmacy, Komarapalayam, Namakkal Dt, Tamilnadu - 638 183, India.
[3]UG Scholars, Excel College of Pharmacy, Komarapalayam, Namakkal Dt, Tamilnadu - 638 183, India.

ABSTRACT

Phytosomes of neem leave extract has been successfully formulated in this procedure firstly *Azadirachta indica* leaves were morphological Characterized. Neem leave powder was subjected for various physiochemical analysis and extracted out methanolic fraction of neem leaves, yield of the extract which was 6.93%. Phytochemical screening found that carbohydrate, alkaloids and flavonoids were present. Phytosome formulation was formed using different ratio of Phosphatidylcholine and Cholesterol (4:1). This formulation was characterized under various parameters like yield, drug content and encapsulation efficiency. The formulation yield was 71.15 %, drug content was 88.11% and encapsulation efficiency was 72.31%. Phytosome formulation was incorporated with gel and evaluated under the various parameters, pH 6.6, viscosity 98 centipoises (cp) of formulation was observed and spreadability between 5.6cm.

Keywords: *Azadirachta indica*, Phytosome, Physiochemical analysis, Gel.

INTRODUCTION

Novel herbal drug carriers cure particular disease by targeting exactly the affected zone inside a patient's body and transporting the drug to that area [1]. Novel drug delivery system is advantageous in delivering the herbal drug at predetermined rate and delivery of drug at the site of action which minimizes the toxic effects with the increase in bioavailability of the drugs [2]. Phytosomes are novel complexes which are prepared by reacting from 3-2 moles but preferably with one mole of a natural or synthetic phospholipid such as phosphatidylcholine, phosphatidyl ethanolamine or phosphatidyl serine with one mole of component for example flavolignanans [3], either alone or in the natural mixture in aprotic solvent such as-dioxane or acetone from which complex can be isolated by precipitation with non-solvent such as aliphatic hydrocarbons or lyophilisation or by spray drying. In the complex formation of phytosomes the ratio between these two moieties is in the range from 0.5-2.0 moles [4, 5]. A gel is a two-component, cross linked three-dimensional network consisting of structural materials interspersed by an adequate but proportionally large amount of liquid to form an infinite rigid network structure which immobilizes liquid continuous phase within [6, 7].

Neem is a very beneficial plant that is used to cure many diseases [8]. Every part of the neem tree like neem seed, leaves, bark, roots and twigs can be used for medicinal purposes [9].

For better and improved bioavailability, natural phytoconstituents must have a good balance between hydrophilicity and hydrophobicity. This is achieved through phytosome technology.

Corresponding Author:- **Dr. Vasuki K** Email: 007vasuki@gmail.com

MATERIALS AND METHODS

PRELIMINARY WORK

Collection of Plant material

Leaves of *Azadirachta indica* was collected in the month of January from Namakkal, Tamilnadu, India.

Drying and Size Reduction of Plant Material

Leaves of *Azadirachta indica* were dried under shade in laboratory. They were pulverized to make coarse powder. The coarse powder of leaves was passed through sieve No. 18 to maintain uniformity and stored in cool and dry place for study.

Screening of Powder (Physiochemical Analysis)

Physiochemical screening of powdered leaves under the parameters Loss on Drying, Total Ash Value, Acid Insoluble Ash Value, Water Soluble Ash Value and Foaming Index was done by the standard methods.

Preparation of *Azadirachta indica* leaves extract

(a) Extraction of leaves of *Azadirachta indica* was done by Soxhlet extraction method.

(b) Soxhlet Extraction: Soxhlet apparatus was used for the solvent extraction and methanol was selected as a solvent for extraction while petroleum ether was used for defatting of waxy materials.

Phytochemical Screening

Extract obtained after extraction were subjected for phytochemical screening to determine the presence of following various phytochemical present in the extracts.

Preparation of Phytsomes of *Azadirachta indica* extract

Accurately weighed quantity of phosphatidylcholine and cholesterol were dissolved in 10ml of chloroform in round bottom flask and sonicated for 10min using bath sonicator. Organic solvent removal is done by Rotary evaporator (45-50°C). After complete removal of solvent thin layer of phospholipids mixture was formed. This film was hydrated with methanolic extract of neem leaves in rotary evaporator (37-40°C for 1hour). After hydration, mixture of lipid and plant extract was sonicated for 20min in presence of ice bath for heat dissipation. Then prepared phytosomes were filled in amber colored bottle and stored in freezer (2-8^{0}C) until used.

Characterization of Phytsomes of *Azadirachta indica* extract

Drug Content & Encapsulation Efficiency

20mg of the microspheres from each batch were taken and digested in 100 ml of 0.1N HCl in a 100 ml volumetric flask and kept aside with intermittent shaking for 24 h. Then, the contents of the flask were filtered by using Whatman filter paper no.1. Then 1 ml of the filtrate was diluted with 50ml of Dimethyl Sulfoxide (DMSO) in a volumetric flask and sonicated for 10min so that leave out neem extract from phytosome. This was again filtered by using Whatman filter paper no.1; one ml from this was further diluted with methanol up to 10ml and absorbance measured at 330nm using methanol as blank. After recording the absorbance, the drug content and encapsulation efficiency were calculated. The readings were taken thrice and the average reading was taken for further calculation.

Preparation of Neem extract loaded phytosomal gel

Dissolve different concentrations of HPMC in ethanol and propylene glycol in water were mixed together using a magnetic stirrer, at 25 rpm. The Neem extract loaded phytosome was poured into the polymer solution. The solution was kept under stirring and then the pH was adjusted using 0.1M NaOH and the formulated gel was taken for further analysis.

Evaluation of prepared Gel

Physical examination

The formulation was manually examined to check any variations in the color, odor, andtexture.

Determination of pH

pH of each formulation was determined by using pH meter which was calibrated before with buffer solutions of pH 4, 7 and 9.

Determination of Viscosity

Viscosity of each formulation was determined using Brookfield viscometer with spindle at room temperature and at 5, 10, 20, 50 and 100 rpm.

Drug content

0.2gm of the gel formulation (equivalent to 10mg of drug) was taken in 100ml volumetric flask which contains 20ml of phosphate buffer pH 7.4 and sonicated for 15 minutes. Volume was made upto 100 ml. 1ml of above solution was further dilute to 10ml by using phosphate buffer of pH 7.4. The resultant solution was subjected to UV spectrophotometric analysis at 330nm and the absorbance was noted down.

Spreadability

To determine spreadability of the gel formulations, two glass slides of known standard dimensions are selected. Formulation whose spreadability to be determined was place on one slide and then other slide was kept over its top such that the gel is sandwiched between the two slides. The slides were pressed upon each other so as to displace any air present, and the adhering gel was wiped off. The two slides were placed onto a stand such that only the lower slide is held firm by the one opposite fangs of the clamp clips and allows the upper slide to slip freely over it by the force of weight tied

Tie the 20-gm weight to the upper slide carefully. The time taken by the upper slide to completely detach from the lower slide was noted. The spreadability was calculated by using standard formula.

Stability study

Stability study is performed for the formulation shows greatest drug release and hence can be termed as 'best formulation' from within those that are developed. Stability study was carried out for 1 month; the formulation was kept in stability chamber at 40^{o}C and at 75% relative humidity and 4^{o}C. After one month the formulation was checked for parameters like phase separation pH and drug content.

RESULTS

Morphological Characterization of *Azadirachta indica* leaves

Leaves of *Azadirachta indica* were green in color, bitter in taste, Length - 1.5 to 3cm,Width -1to1.5cm in size, ovate in shape and Rough outer periphery.

Physiochemical analysis of *Azadirachta indica* leaves powder.

Extract of *Azadirachta indica*

Extractive values of Petroleum ether extracts of Azadirachta indica were % Yield (2.91% w/w), Dark green color, greasy in Consistency and methanol extracts of Azadirachta indica were % Yield (6.34% w/w), Dark green color, semi solid consistency.

Table 1: Physiochemical analysis of powder of Azadirachta indica leaves.

S.NO.	PARAMETERS	OBSERVATION
1	Total ash value	9g
2	Loss on drying	1.2g
3	Acid insoluble ash value	2.9g
4	Water soluble ash value	1.6g
5	Foaming index	6 ml

Table 2: Phytochemical screening of methanolic extract of Azadirachta indica leaves.

S.NO.	PARAMETERS	OBSERVATION
1	Carbohydrates	
	Molisch's Test	(+)
	Fehling's Test	(-)
	Benedict's test	(+)
2	Tannins	
	With 5%ferric chloride solution	(+)
	With 10% aqueous Potassium dichromate solution	(-)
	With 10% lead acetate solution	(-)
3	Alkaloids	
	Dragendorff's Test	(-)
	Mayer's Test	(-)
	Hager's Test	(+)
4	Glycosides	
	Borntrager's Test	(+)
	Legal Test	(-)
	Baljet Test	(+)
5	Flavonoids	
	Shinoda's Test	(+)
	Alkaline reagent test	(+)
	Lead test	(+)
6	Steroids and Sterols	
	Libermann-Burchard Test	(+)
	Salkowski Test	(-)
7	Proteins and Amino Acids	
	Biuret Test	
	Ninhydrin Test	(+)
	Millon's Test	(-)
		(+)

Formulation of Neem extract loaded Phytosome

Table 3: Formulation of Neem extract loaded Phytosome.

Neem leaves extract (mg)	Phosphatidylcholine (PC) (mg)	Cholesterol (CL) (mg)	Phytosome PC: CL
200	100	25	4:1

Characterization of Neem Extract Loaded Phytosome

Table 4: Characterization of Neem Extract Loaded

Yield (%)	Drug Content(%)	EncapsulationEfficiency (%)
89.12 ± 0.05	90.21 ± 0.21	78.67 ± 1.52

Formulation of Topical Gel of Neem Extract Loaded Phytosome

Table 5: Formulation of Topical Gel of Neem Extract Loaded Phytosome

Formulation	Quantity
Phytosome	0.4GM
HPMC	0.5GM
Ethanol (ml)	5ML
Propylene glycol	1GM
Distilled water	1ML

Physical Evaluation of Neem Extract Loaded Phytosome gel

Table 5: Physical Evaluation of Neem Extract Loaded Phytosome gel

Clarity	Odor	Phase Separation	Washability	Homogeneity	Grittiness
Clear	No	No	Washable	Yes	No

Evaluation of Neem Extract Loaded Phytosome gel

Table 6: Evaluation of Neem Extract Loaded Phytosome gel

pH	Spreadability (cm)	% DrugContent	Viscosity(cp)	% Permeation
6.9	5.6 ± 0.3	99.9 ± 1.2	110 ± 1.8	83.2%

Stability study of Neem extract loaded Phytosome gel

Table 7: Stability of Neem extract loaded Phytosome gel.

Phase separation		pH		Drug content (%)	
4^oC	40^oC	4^oC	40^oC	4^oC	40^oC
No	No	7.1	7.3	100 ± 1.6	98 ± 1.6

DISCUSSION

Phytosomes of neem leave extract has been successfully formulated in this procedure firstly *Azadirachta indica* leaves were morphological Characterized. Neem leave powder was subjected for various physiochemical analysis like total ash value, Loss on Drying, Acid Insoluble Ash Value, Water Soluble Ash Value and Foaming Index and extracted out methanolic fraction of neem leaves, yield of the extract which was 6.34 %. Phytochemical screening found that carbohydrate, alkaloids and flavonoids were present.

Phytosome formulation was formed using ratio of Phosphatidylcholine and Cholesterol (4:1). This formulation was characterized under various parameters like yield, drug content and encapsulation efficiency. Phytosome formulation was incorporated with gel and formed clear, odorless, washable, homogeneous, stable and free from grittiness gel was evaluated under the various parameter, this formulation was characterized under various parameters like yield, drug content and encapsulation efficiency. The formulation yield was 71.15 %, drug content was 88.11% and encapsulation efficiency was 72.31%. Phytosome formulation was incorporated with gel and evaluated under the various parameters, pH 6.6, viscosity 98 centipoises (cp) of formulation was observed and spreadability between 5.6cm.

CONCLUSION

Neem leaves collected and extracted under soxhlet apparatus, physicochemical and phytochemical analysis was performed and characterized the drug and phytosome was formulated and phytosome was evaluated on various parameter like visuality, drug content and encapsulation efficiency. Phytosome was incorporated into gel and evaluated under gel parameter like clarity, grittiness, spreadability and drug content. In this present study, we planned preparation of phytosome allows for the achievement of an improved formula of bioactive

compounds extracted from neem leaves for greater bioavailability. Moreover, the natural active compounds have lower adverse effects compared to synthetic products and thus they can be used safely for a longer period of time.

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