

Preparation and Evaluation, of Neem Loaded Nanophytosomes

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ABSTRACT:

Nanophytosomes represent a high-level advance in phytosome technology, which is needed to enhance the absorption, duration and overall efficacy of healthful components taken from plants. Phytosomes are vesicular lipid carriers which join plant-derived phytoconstituents to phospholipids to enhance their bioabsorption and effectiveness. On the basis of phytosome technology, nanophytosomes possess a nanoscale structure between 50-500 nm diameter and yet possess novel physicochemical properties and superior permeation capabilities across membranes.

Inspired by the inadequacy of traditional plant extracts like those with poor water solubility, weakly permeable through the membranes, and quickly degraded, the nanophytosomes were developed. Sealing phytochemicals in a lipophilic nanometric lipid bilayer gives rise to nanophytosomes with improved solubility, controlled release, and greater resistance to environmental exposure. As a result of these properties, nanophytosomes become an attractive tool for the delivery of various bioactive compounds including polyphenols, flavonoids, alkaloids and essential oils.

Synthesis of nanophytosomes can be done by employing multiple options – solvent evaporation, thin-film hydration and microemulsion, and each of the alternatives brings its improvement in particle size management, encapsulation efficiency, and potential of scaling up. The choice of phospholipids, surfactants, and preparation parameters has a significant influence on the size, charge and to what degree the target compounds are protected in the obtained nanophytosome.

Keywords: Nanophytosomes, Phytoconstituents, Antimicrobial.

INTRODUCTION:

Neem tree, botanically *Azadirachta indica* is a fast-growing evergreen species with a widespread distribution on the Indian subcontinent but is cultivated in tropical and subtropical regions throughout the world. Meliaceae family contains neem as a plant used for its impressive human health, agricultural practices, and ecological balance contributions. This tree grows well has a variety of soils and it is especially appropriate for dry climates. According to Ayurveda known to use neem for its leaves, bark,

seeds and oil because of their antimicrobial, anti-inflammation and healing qualities. Apart from its use in medicine, neem also contains previously natural insecticidal qualities that can be used safely for pest control as opposed to the traditional use of pesticides. Its versatility of applicability and sustainable orientation have led to neem being perceived as "Nature's Pharmacy" and "Village Pharmacy" in the culture of many people.[7]



Figure 1 : *Azadirachta Indica* Leaves

MATERIALS AND METHOD:

Material :

Soy Lecithin (Amepurva Forums Nirant Institute of Pharmacy), Dichloromethane (Amepurva Forums Nirant Institute of Pharmacy),
Phospholipids (Amepurva Forums Nirant Institute of Pharmacy) ,
Ethanol (Amepurva Forums Nirant Institute of Pharmacy).

Method of preparation :

• Maceration Process and Authentication

Fresh neem leaves were collected and washed thoroughly. Shade-dry the leaves for 3–5 days until they become brittle.

Grinded the dried leaves into coarse powder using a grinder.

Weighed the neem leaf and transferred it to a clean container. Later added the neem leaves into the ethanol (solvent).

Cover the container and let the mixture stand at room .

• Solvent Removal & Drying

Later , the obtained extract was kept on water bath to remove the excess amount of water present in the paste.

The obtained extract was kept on butter paper under the sunlight for 2 days to allow it for the complete drying of it .

Once the dried extract was obtained, it was grinded it into a fine powder.

The powder obtained was sieved to avoid any lumps in it.

• Preparation of Neem Nanophytosomes

Phytosomes were prepared by using lyophilization method with different molar ratio of Azadirachta indica and phosphatidylcholine. Azadirachta Indica, phosphatidylcholine and cholesterol were dissolved in a mixture of ethanol. Later to this mixture cholesterol dissolved in dichloromethane was added into bottom flask and mixed properly. Later, the solution was poured into the petri dish and was kept to drying for 48-72 hours.

Formulation Table of Nanophytosomes of Azadirachta Indica.

Batch number	Azadirachta Indica	Phosphatidylcholine	Cholesterol
F1	1	2	0.2
F2	1	2	0.4
F3	1	2	0.8
F4	1	2	1.6

Evaluation of Nanophytosomes

I. Entrapment efficacy :

For assessing the effectiveness of a formulation, in this case, neem (Azadirachta Indica) nanophytosomes, the term 'Entrapment efficacy' (EE) serves as one of the parameters. EE evaluates the proportion of the active constituents which in a case of neem is Azadirachtin that gets entrapped within the nanophytosome system.

$EE (\%) = \frac{\text{Amount of drug in NP (mg)}}{\text{Amount of drug added (mg)}} \times 100.$

Batch	% Drug efficacy
F1	95.20
F2	96.45
F3	93.48
F4	97.50

II. Particle size analysis

Sample is diluted in the proportion of 1: 10 in methanol. Sonicated for 10 minutes and filtered through Whatman filter paper. Filtrate is taken for analysis. Light from the laser light source illuminates the sample in the cell. The scattered light signal is collected with detectors, at a 90 degree (right angle). Keep sample in cuvette. Go to condition set and fill

name of sample, Run time for 120 seconds and select glass cuvette as sample holder. Start measurement and view the report in nanometers for particle size.

Determination of Nimbidin :

Prepare a solution of the test sample (neem oil or extract) in ethanol. Introduce a couple of drops of sodium hydroxide (NaOH 5%) to the ethanol solution sample. The existence of nimbidin, which is indicative phenol, is confirmed by the yellow coloration formed.

Determination of Polysaccharides :

Mix the sample with anthrone reagent (anthrone in sulfuric acid). Heat gently in a boiling water bath For-

mation of a green to blue-green color indicates the presence of polysaccharides.

Conclusion:

1. Particle Size : The particle size of all formulated batch have size range of nanometer. So all the batches pass the test.
2. The % entrapment efficiency of formulation batch of 2,3,4 are 93.81, 91.16, 95.75 respectively .
3. Antimicrobial Assay:- The extract of Azadirachta Indica leaves demonstrated efficacy against Bacterial and fungal strains in the current study. According to the antibacterial activity Profile, leaf extracts from Azadirachta Indica exhibited activity against Gram negative (E. coli) And Gram positive (Staphylococcus Aureus) bacteria.



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