“FORMULATION AND EVALUATION OF HERBOSOMES AND ITS ANTIMICROBIAL ACTIVITY”

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ABSTRACT

Herbosomes are a relatively new technology that involves encapsulating herbal extracts in liposomes, which are tiny spheres made of phospholipids. This allows for better absorption of the herbal compounds into the body. Herbosomes have a higher bioavailability compared to traditional herbal extracts, improved stability, and can be designed to target specific areas of the body, as well as reduced side effects as they can be delivered in smaller doses. This study was to develop and evaluate a novel herbosome formulation and its anti microbial activity. The technique of thin-film hydration was utilized, with soya-lecithin serving as the phospholipid of choice. The herbosome was confirmed by taking into account a number of factors, including as particle size, zeta-potential, scanning electron microscopy and its anti microbial activity. The optimized technique provided that particle size within the range (210.3 nm to 795.2 nm) and good polydispersity index, an average Zeta potential (-0.1 mV to -1.2 mV), and Shape and morphology analysis using a scanning electron microscope revealed a smooth surface and spherical shape. The result of antibacterial activities showed that Herbosome had an inhibiting activity against *E.coli.*. As conclusion, the formulated herbosomes was showed promising results to be developed as a new antibacterial formulation.

Keywords: Herbosome, polydispersity index, thin-film hydration, *E.coli*

1. INTRODUCTION

Herbosomes, derived from "HERBO" (plant) and "SOME" (cell-like), bridge Novel Drug Delivery Systems (NDDS) and traditional herbal medicine by complexing phytoconstituents with lipids to enhance permeation and bioavailability of plant extracts. Key challenges in plant extract absorption include lipophilicity and complex molecular structures, which can lead to poor in vivo performance despite excellent in vitro bioactivity. Many plant extracts suffer from low lipid solubility and inappropriate molecular size, resulting in inadequate absorption, low bioavailability, and degradation in gastric fluids when taken orally.

Herbosomes address these issues by reacting phospholipids (natural or synthetic) with selected botanical constituents in an appropriate solvent, forming a novel entity. The choline part of the phospholipid binds to hydrophilic chief active constituents, while the lipid-soluble phosphatidyl part attaches to the choline-bound complex, creating a stable and bioavailable lipid complex **(Manach *et al.*, 2004)**.

The formation of phyto-phospholipid complexes can be confirmed using FTIR spectroscopy, X-ray diffraction, NMR, and molecular imaging, with drug content quantified by HPLC. Additional techniques include Dynamic Light Scattering (DLS) with a computerized inspection system and Photon Correlation Spectroscopy for particle size and Zeta potential determination, and Ultracentrifugation for assessing entrapment efficiency. This technology promises to enhance the effectiveness of plant-based medicines by overcoming the limitations of traditional herbal extracts **(Bhattacharya *et al.*, 2009)**.

2. MATERIALS AND METHODS

2.1 Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Oxford Fine-Chem Chem. Ltd. (Mumbai, India), Sunchem and SRL Pvt. Ltd. (Mumbai, India). The investigation only utilised analytical-grade compounds.

2.2 Formulation of extract loaded herbosome formulation

Herbosome are basically herbal-liposomes specially designed to enhance the bioavailability of the herbals so that they have targeted site of action. In this study, we have designed Herbosome by using conventional method of preparation i.e., thin film hydration (TFH) method describe with few modifications. Briefly soya lecithin and cholesterol were dissolved in different concentration in chloroform-methanol and 200 mg *Annano squamosa* extract was added in the solution, then the mixture was evaporated in a rotary evaporator. The thin film formed in the round-bottomed flask was hydrated by adding phosphate buffer 7.4. The suspension was stirred by magnetic stirring for 30 min and then sonicated for few hours. Herbosome was then successfully collected in vessels and used for further drug development **(Moghimipour, E., & Handali, 2012).**

Table 1: Composition of Herbosome formulation

Ingredients	Formulation Code				
	F1	F2	F3	F4	F5
Soya-Lacithin (mg)	50	100	150	200	250
Cholesterol (mg)	300	250	200	150	100
Extract (mg)	200	200	200	200	200
Sonication Time (min)	20	20	20	20	20
Chloroform : Methanol (ml)	20	20	20	20	20
Water	q.s	q.s	q.s	q.s	q.s

2.3 Characterization of Herbosome

1. Particle size

The size of herbosome was measured using Malvern Zeta sizer (Malvern Instruments) **(Singh and Vingkar 2008).**

2. Zeta potential

The zeta potential was measured by Zetasizer Malvern instruments **(Đorđević *et al.*, 2015).**

3. Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the extract loaded herbosome were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pretreated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons **(Anwer *et al*., 2019).**

4. Antimicrobial activity

Well Diffusion Assay

The bacterial suspension of *E. coli* was standardized to 10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate **(Mohammadi-Sichani *et al*., 2012).** The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the herbosome (0.5, 1, 1.5 and 2mg/ml) formulation solution. 100 µl of the sample was loaded. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37^o C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well **(Manandhar *et al*., 2019).**

3. RESULTS

3.1 Characterization of Herbosomes

1. Particle Size

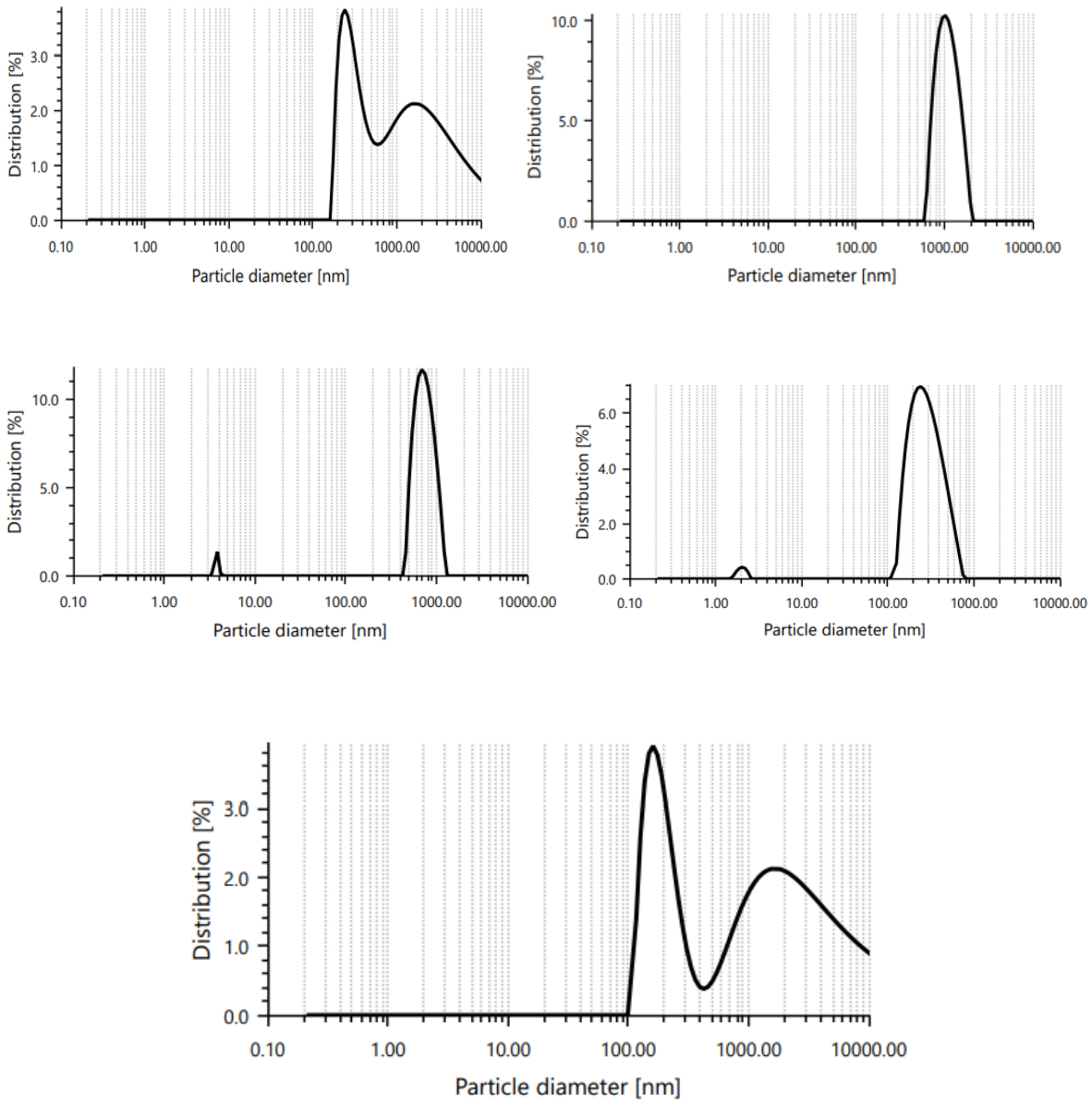

Figure 1: Particle size (F1 to F5)

2. Zeta potential

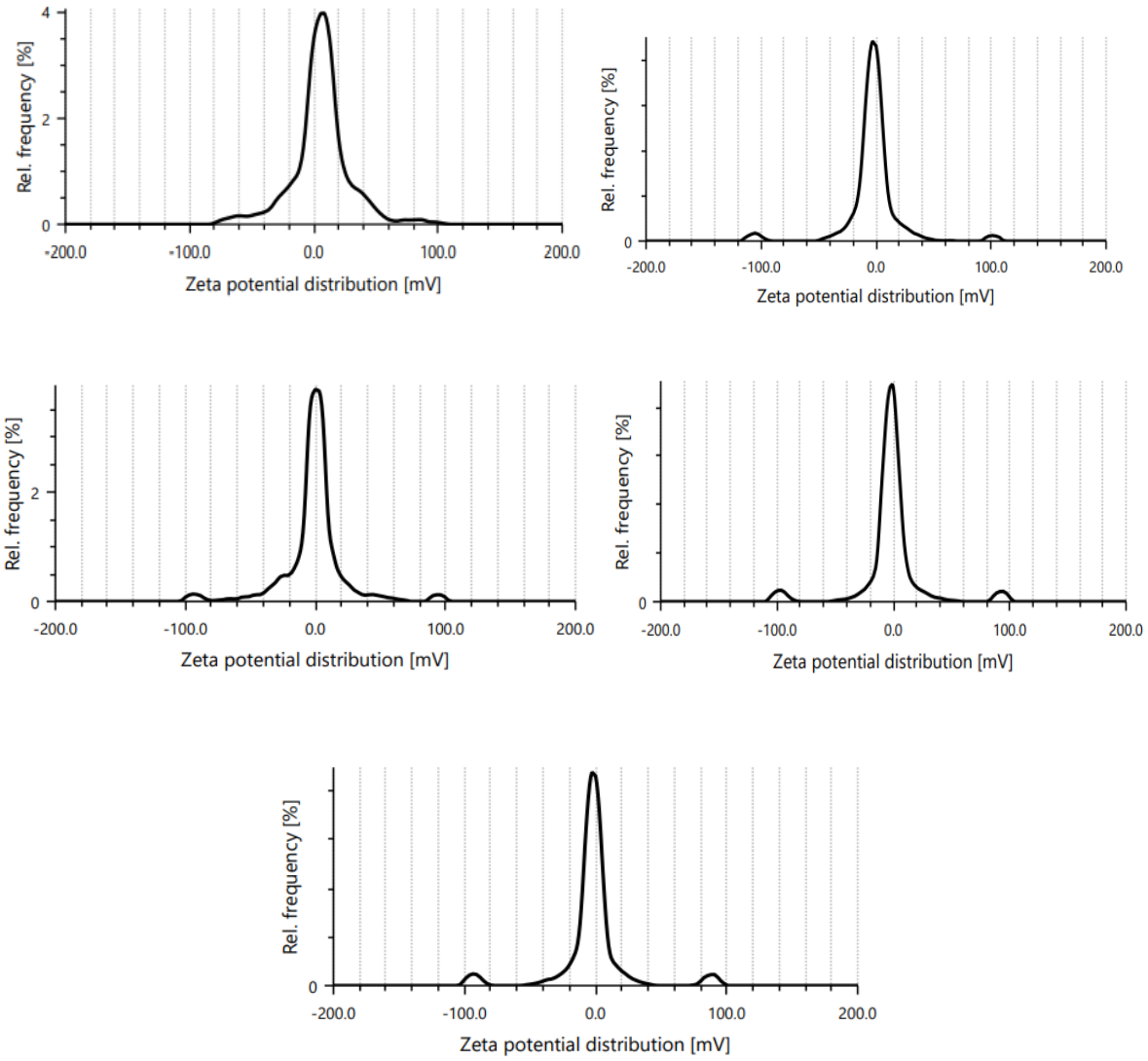

Figure 2: Zeta potential (F1 to F5)

Table 2: Particle size and Zeta potential

S. No	Formulation Code	Particle size (nm)	Zeta potential
1.	Herbosomes F1	303.8 nm	-0.3 mV
2.	Herbosomes F2	795.2 nm	-0.1 mV
3.	Herbosomes F3	751.8 nm	-1.2 mV
4.	Herbosomes F4	297.4 nm	-0.5 mV
5.	**Herbosomes F5**	**210.3 nm**	**-0.2 mV**

Discussion

The particle size and Zeta potential is one of the most important parameters for the characterization ofherbosomes. The average particle size and Zeta potential of the prepared extract loaded herbosomes was measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of extract loaded herbosomes was found to be range 210.3 nm to 795.2 nm. Zeta potential of all formulations was found to be range -0.1 mV to -1.2 mV with peak area of 100% intensity. These values indicate that the formulated herbosomes are stable.

3. Scanning electron microscope (SEM)

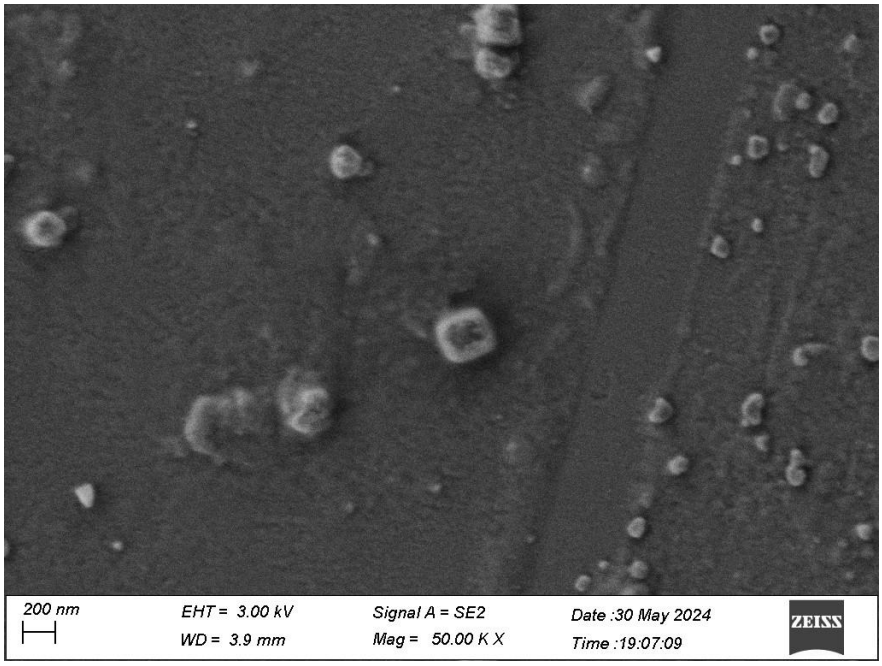

Figure 3: SEM of F5 Formulation

Discussion

Herbosomes were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared herbosomes at 50.00 KX magnification showed that the herbosomes were porous with a smooth surface morphology and spherical shape. The porous nature of herbosomes was clearly observed in the SEM images.

3.2 Results of antimicrobial activity of all formulations

Table 3: Antimicrobial activity of formulation

S. No	Sample name	Zone of Inhibition (mm)
1	Herbosomes (0.5mg/ml)	10 mm
2	Herbosomes (1mg/ml)	12 mm
3	Herbosomes (1.5mg/ml)	13 mm
4	Herbosomes (2mg/ml)	15 mm

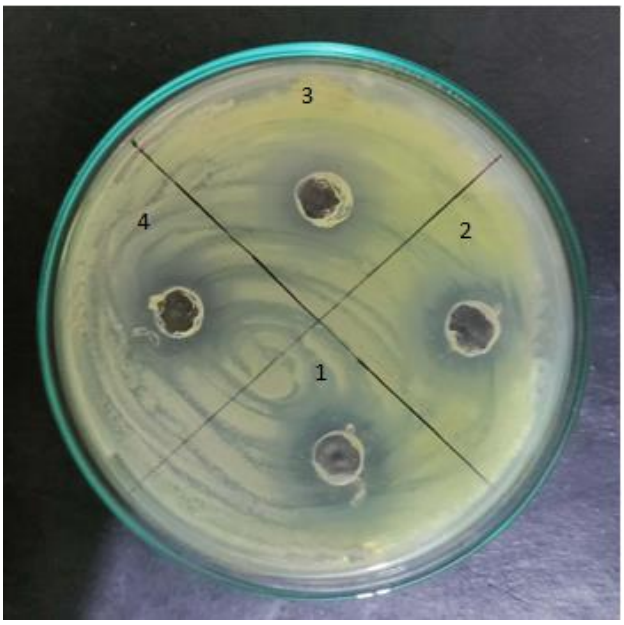

Figure 4: Antimicrobial activity against *E. coli*

4. Summary and conclusion

Herbosomes, formed by loading phytoconstituents into phospholipids, exhibit improved physical stability due to the formation of hydrogen bonds between phospholipids and phytoconstituents. This enhances the absorption of hydrophilic polar phytoconstituents, leading to increased bioavailability and greater therapeutic benefits. Herbosomes represent an innovative formula of botanicals and phytoconstituents, exhibiting enhanced absorption through both oral and transdermal routes when encapsulated with phosphatidylcholine. This technology serves as a bridge between conventional phytoconstituent delivery systems and emerging drug delivery methodologies.

Formulation was carried out by thin film hydration method. Trial batches indicated that polymers are suitable for the *Annano squamosa* herbosomes. Polymer produced good formulations. Soya-Lacithin and Cholesterol were selected for further studies. Scanning electron micrograph of the prepared herbosomes at 50.0 KX magnification showed that the herbosomes were porous with a smooth surface morphology and spherical shape. The porous nature of herbosomes was clearly observed in the SEM images. Particle size and zeta potential was determined by Malvern Zeta sizer. The particle size analysis confirmed that the prepared sample were in the nanometer range. Average particle size obtained for the formulations F1 to F5 were 210.3 nm to 795.2 nm. Zeta potential values of herbosomes indicated that the formulated herbosomes are stable.

Current study indicates for the first time, the potential of herbosomes formulation of *Annano squamosa*extract for enhancing the bioavailability. Moreover, the results demonstrated that the herbosomes based drug delivery approach could be a valuable tool to improve the therapeutic efficacy of phytochemicals by improving their absorption, and bioavailability via altering their physicochemical and release properties.

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