

**FORMULATION AND EVALUATION OF HERBOSOMES OF ANNONA SQUAMOSA
LEAF EXTRACT AND ITS ANTIMICROBIAL ACTIVITY****Vishal Deshmukh^{*1}, Rahul Dubey², Dr. Bala Subramanian³**¹Student – Millennium College of Pharmacy and Science, Bhopal.²Associate Professor - Millennium College of Pharmacy and Science, Bhopal.³Dean - Millennium College of Pharmacy and Science, Bhopal.***Corresponding Author: Vishal Deshmukh**

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ABSTRACT

The present study was undertaken to formulate and evaluate herbosomes containing *Annona squamosa* leaf extract as a novel herbal drug-delivery system and to investigate their antimicrobial potential. The leaves were extracted using petroleum ether and methanol, and the percentage yield was found to be 0.52% and 2.13%, respectively. Preliminary phytochemical screening of the extracts revealed the presence of various bioactive constituents, including alkaloids, glycosides, carbohydrates, proteins, flavonoids, phenolic compounds, saponins, triterpenoids, and steroids, with the methanolic extract showing a broader phytochemical profile. Solubility studies indicated that the extract was freely soluble in methanol and soluble in ethanol, dimethyl sulfoxide, and water. The extract was subsequently incorporated into herbosomes, and five formulations (F1–F5) were prepared and characterized for their physicochemical properties. Particle-size analysis revealed sizes ranging from 210.3 to 795.2 nm, with formulation F5 exhibiting the lowest particle size of 210.3 nm. Zeta-potential values ranged from –0.1 to –1.2 mV. Scanning electron microscopy of the optimized F5 formulation revealed spherical particles with a smooth and porous surface morphology. The antimicrobial activity of the herbosomal formulation was evaluated against *Escherichia coli* using the agar diffusion method. The formulation demonstrated concentration-dependent antimicrobial activity, with zones of inhibition of 10, 12, 13, and 15 mm at concentrations of 0.5, 1, 1.5, and 2 mg/mL, respectively. The findings demonstrate that *Annona squamosa* leaf extract can be successfully incorporated into a herbosomal delivery system and that the developed formulation possesses promising antimicrobial activity against *E. coli*. Herbosomal delivery may provide a useful approach for improving the pharmaceutical potential and delivery of bioactive constituents present in *Annona squamosa* leaves.

KEYWORDS: *Annona squamosa*; Leaf extract; Herbosomes; Phytochemical screening; Particle size; Zeta potential; SEM; Antimicrobial activity; *Escherichia coli*; Herbal drug delivery.**1. INTRODUCTION**

Medicinal plants have been widely used as therapeutic agents because they contain diverse bioactive compounds with pharmacological properties. Phytoconstituents such as flavonoids, phenolics, alkaloids, tannins, saponins, glycosides, and terpenoids have demonstrated antioxidant, anti-inflammatory, antimicrobial, and other biological activities. However, the therapeutic application of plant extracts may be limited by poor aqueous solubility, low bioavailability, instability, and inadequate delivery of active constituents to the site of action (Latif and Nawaz, 2026).

Annona is the second largest genus of flowering plants in the family Annonaceae family. *Annona squamosa* Linn, commonly known as sugar apple, is the most developed tropical fruit in the family. During this time, custard apple only known for its edible fruit, though various parts of *Annona squamosa* L. are traditionally used for treatment of various diseases (Qi et al., 2025). *Annona squamosa* seeds extracts are reported to have anti-tumor activities against human hepatoma cells, the petroleum ether extract from the bark of *Annona squamosa* is studied for its analgesic and anti-inflammatory activity, the ethanolic extract of *Annona squamosa* leaves has been reported to have antidiabetic effect, the aqueous

extracts of *A. squamosa* leaves show antifungal and antioxidant activities. Therefore, this study is conducted to evaluate the antibacterial activity of various concentration ethanolic leaf extract of *Annona squamosa* L. against the growth of *Escherichia coli* (Shokr *et al.*, 2024).

The Ethnobotanical record reported the evokes obtained from various parts of the *Annona squamosa*, have been employed and used in conventional pharmacological applications in different countries to cure a variety of diseases, such as insecticidal, an anti-tumour agent, anti-diabetic, antioxidant, antilipidemic and anti-inflammatory agent, epilepsy, diarrhoea, heart issues, parasitic infections, constipation, bleeding, fever, and ulcers (Singh *et al.*, 2025). Pruning of *Annona squamosa* generates large amounts of leaves, posing disposal issues for farmers. However, these leaves hold great value due to their rich bioactive content such as glycosides, flavonoids, alkaloids, and phenols which contribute to antioxidant, antimicrobial, antidiabetic, anticancer, and hepatoprotective properties (Parsana *et al.*, 2023).

The word "herbo" refers to plants, while "some" implies a cell-like structure. Most biologically active compounds found in plants are polar or water-soluble in nature. However, these water-soluble phytoconstituents such as flavonoids, tannins, and glycoside aglycones — often show poor absorption in the body. This is mainly due to their large molecular size, which prevents passive diffusion, or their low lipid solubility, which limits their ability to pass through lipid-rich biological membranes, ultimately resulting in low bioavailability. Herbosomes represent an advanced herbal formulation, where plant-derived active compounds are combined with phospholipids (Singh *et al.*, 2023). This combination significantly improves their absorption and bioavailability in the body, leading to enhanced therapeutic effectiveness compared to conventional herbal extracts or isolated compounds. As a result, herbosome technology helps to overcome the limitations associated with traditional herbal therapies (Joshi *et al.*, 2024).

These extracts often contain not only the desired active compounds but also unwanted or potentially toxic substances. Advances in phytochemistry and analytical techniques have made it possible to isolate and extract specific active ingredients or groups of related compounds from plants for therapeutic use (Altemimi *et al.*, 2017). This reduced bioavailability is mainly due to the complex, multi-ring structures of the active plant compounds, which prevent their absorption into the bloodstream through simple passive diffusion. Additionally, many bioactive phytoconstituents are water-soluble, and their limited lipid solubility restricts their ability to cross lipid-rich biological membranes. When taken orally, these compounds can also be broken down or inactivated by the acidic gastric environment, or

their effectiveness may be reduced due to interactions with other drugs or nutraceuticals (Patel *et al.*, 2020).

Therefore, the present study was designed to formulate and evaluate herbosomes containing *Annona squamosa* leaf extract and investigate their antimicrobial activity against *Escherichia coli*. The study includes extraction and phytochemical screening of the leaves, evaluation of solubility, preparation of different herbosomal formulations, characterization by particle-size analysis, zeta-potential measurement and scanning electron microscopy (SEM), followed by in vitro antimicrobial evaluation. The developed formulation may provide a promising herbal delivery approach for the utilization of *Annona squamosa* as a potential source of natural antimicrobial agents.

2. MATERIALS AND METHODS

2.1 Chemicals

Glacial Acetic Acid, Ammonia, Nitroprusside and Sodium Hydroxide was obtained from Merck. 95% Alcohol, Conc. HCl and Chloroform were procured from Clorofiltind, while Conc. H₂SO₄ was supplied by Fizmerck. Ethanol, was obtained from Molychem. Research lab provided the Petroleum ether. 1% Copper Sulphate Solution was obtained from Rankem. Himedia were procured from Magnesium. Soya-Lacithin and Cholesterol was supplied by Oxford fine chem. All other reagents and solvents used were of analytical grade.

2.2 Plant collection

The medicinal plant *Annona squamosa* (300 gm) was collected. After cleaning, plant parts (leaf) were dried under shade at room temperature for 3 days and then in oven dried at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration.

Authentication of selected traditional plant - Medicinal plant *Annona squamosa* was authenticated by a plant taxonomist in order to confirm its identity and purity. Plant authentication no-208/Saif./Sci./Clg/Bpl.

2.3 Extraction

In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *Annona squamosa* was placed in thimble of soxhlet apparatus. Soxhlation was performing at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dry and afterward re-extracted with methanol solvent. For each solvent, soxhlation was continued till no visual colour change was observed in siphon tube and completion of extraction was confirmed by absence of any residual solvent, when evaporated. Obtained extracts were evaporated using rotary vacuum evaporator (Buchtype) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula.

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts were observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use (Baidya *et al.*, 2002).

2.4 Solubility study

Qualitative solubility of *Annano squamosa* in different solvents was determined according to USP NF, 2007. Approximately 1 mg of *Annano squamosa* was weighed and transferred into a 10 ml test tube and dissolved in the respective solvents (1 ml each of methanol, DMOS ethanol, and water) (Jain *et al.*, 2020).

2.5 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 (given in table 4) 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. The various formulation variable considered in this research have been presented. Herbosome was then successfully collected in vessels and used for further drug development (Moghimipour *et al.*, 2013).

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

2.6 Characterization of Herbosome

2.6.1 Particle size

The particle size is one of the most important parameter for the characterization of herbosome. The size of herbosome was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25°C and sample was placed in disposable sizing cuvette (Singh *et al.*, 2008).

2.6.2 Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the herbosome was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-15 minutes before zeta potential measurements (Đorđević *et al.*, 2015).

2.6.3 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. From this interaction between the electron beam and the

specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography (Anwer *et al.*, 2019).

2.7 Antimicrobial activity

2.7.1 Preparation of Nutrient Agar Media

28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121°C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

2.7.2 Well Diffusion Assay

The bacterial suspension of *E. coli* was standardized to 10⁸ CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 10⁸ CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate. 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° 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 (Mohammadi-Sichani *et al.*, 2012).

3. RESULTS AND DISCUSSION

3.1 Percentage Yield

In phytochemical extraction the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used.

Table 2: Percentage Yield of crude extracts of *Annano squamosa* extract.

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
<i>Annano squamosa</i>	Pet ether	297	1.55	0.52%
	Methanol	287.52	6.15	2.13%

3.2 Preliminary Phytochemical study

Table 3: Phytochemical testing of extract.

Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	Methanolic extract
Alkaloids		
Dragendroff's test	+	+
Mayer's reagent test	+	+
Wagner's reagent test	+	+
Hager's reagent test	+	+
Glycoside		
Borntrager test	-	+
Legal's test	-	+
Killer-Killiani test	-	+
Carbohydrates		
Molish's test	-	+
Fehling's test	-	+
Benedict's test	-	+
Barfoed's test	-	+
Proteins and Amino Acids		
Biuret test	+	-
Flavonoids		
Alkaline reagent test	-	+
Lead Acetate test	-	+
Tannin and Phenolic Compounds		
Ferric Chloride test	-	+
Saponin		
Foam test	+	-
Test for Triterpenoids and Steroids		
Salkowski's test	-	+
Libbermann-Burchard's test	-	+

3.3 Solubility study

Table 4: Solubility study of *Annano squamosa*.

Drug	Solvents	Observation/Inference
<i>Annano squamosa</i>	Methanol	Freely Soluble
	Ethanol	Soluble
	DMSO	Soluble
	Water	Soluble

3.4 Characterization of Herbosomes

3.4.1 Particle Size

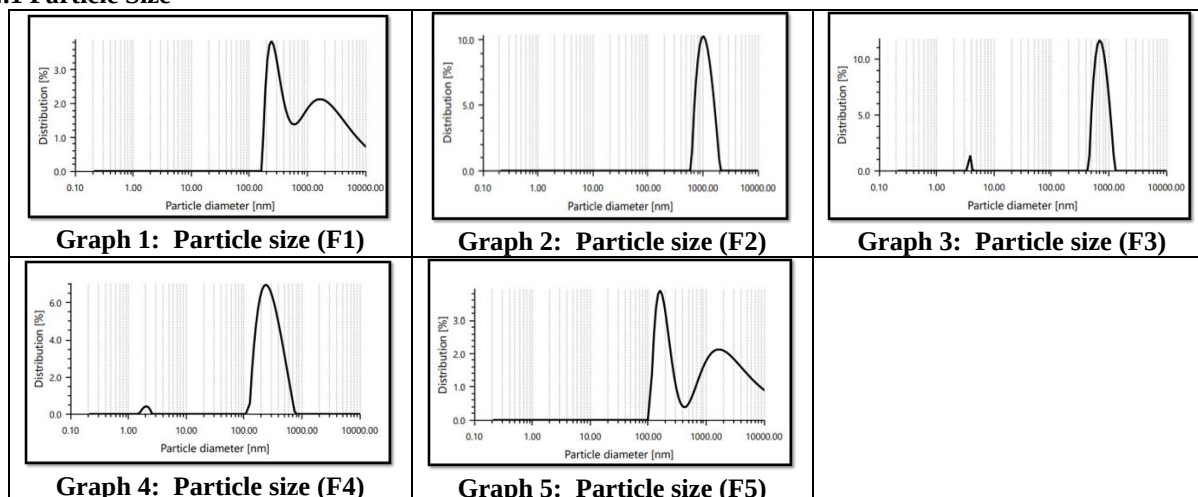


Table 5: Particle size.

Formulation code	Particle size (nm)
Herbosomes F1	303.8 nm
Herbosomes F2	795.2 nm
Herbosomes F3	751.8 nm
Herbosomes F4	297.4 nm
Herbosomes F5	210.3 nm

3.4.2 Zeta potential

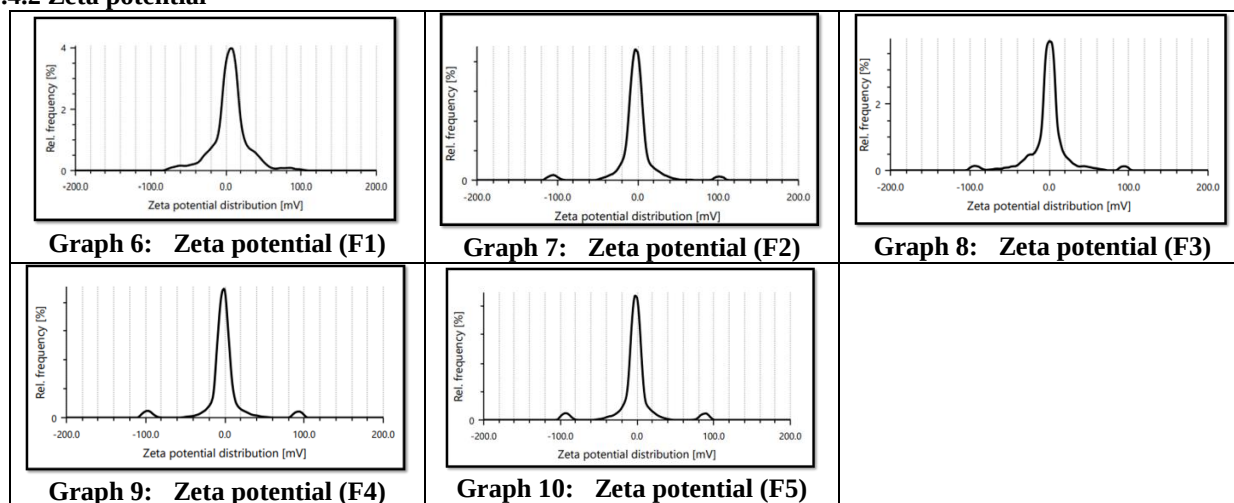


Table 6: Zeta potential.

Formulation Code	Zeta potential
Herbosomes F1	-0.3 mV
Herbosomes F2	-0.1 mV
Herbosomes F3	-1.2 mV
Herbosomes F4	-0.5 mV
Herbosomes F5	-0.2 mV

3.4.3 Scanning electron microscope (SEM)

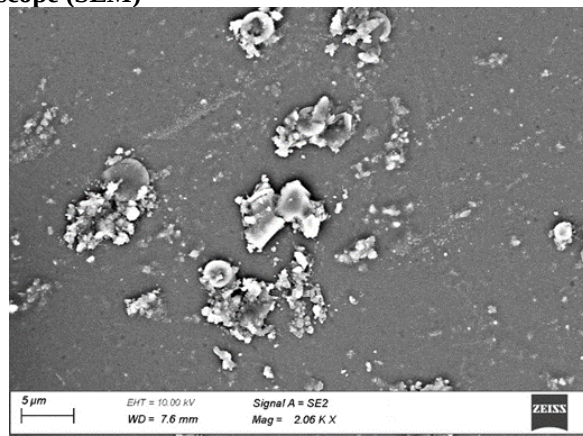


Figure 1: SEM of F5 Formulation.

3.5 Results of antimicrobial activity

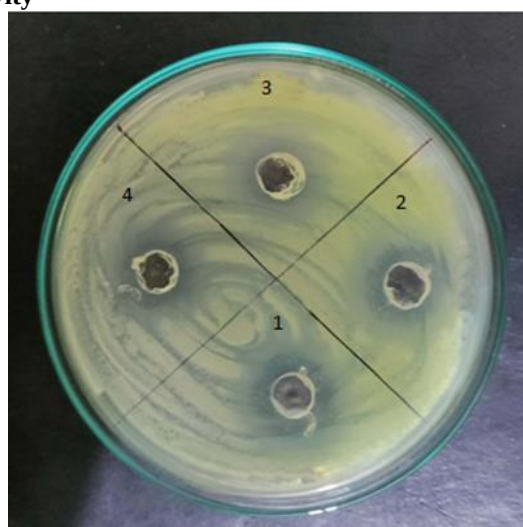


Figure 2: Antimicrobial activity against *E. coli*.

Table 7: Antimicrobial activity of formulation.

Sample name	Zone of Inhibition (mm)
Herbosomes (0.5mg/ml)	10 mm
Herbosomes (1mg/ml)	12 mm
Herbosomes (1.5mg/ml)	13 mm
Herbosomes (2mg/ml)	15 mm

4. CONCLUSION

The present investigation successfully demonstrated the formulation and evaluation of herbosomes containing *Annona squamosa* leaf extract as a potential herbal drug-delivery system. The methanolic extract showed a higher extraction yield (2.13%) than the petroleum ether extract (0.52%) and exhibited the presence of several important phytochemical constituents. The prepared herbosomal formulations showed particle sizes between 210.3 and 795.2 nm, with F5 exhibiting the smallest particle size and favorable morphological characteristics. SEM analysis confirmed the formation of spherical, smooth, and porous particles.

The developed herbosomal formulation also demonstrated concentration-dependent antimicrobial

activity against *E. coli*, with the zone of inhibition increasing from 10 mm at 0.5 mg/mL to 15 mm at 2 mg/mL. These findings suggest that incorporation of *Annona squamosa* leaf extract into a herbosomal system can provide a promising approach for delivering its bioactive phytoconstituents and enhancing its potential antimicrobial application. Overall, the study supports the potential of *Annona squamosa* herbosomes as a novel herbal formulation; however, further studies involving optimization, stability testing, comparative antimicrobial evaluation, toxicity assessment, and in vivo investigations are required to establish their therapeutic applicability.

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