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FORMULATION AND CHARACTERIZATION OF NONIONIC SURFACTANT VESICLE CONTAINING AQUILEGIA FRAGRANS EXTRACT AND ITS ANTI- MICROBIAL ACTIVITY

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ABSTRACT

This study aimed to formulate, develop, and optimize a lipid-based vesicular system (niosomes) containing *Aquilegia fragrans* extract, with emphasis on its antibacterial potential. The plant extract was subjected to percentage yield analysis and phytochemical screening, confirming the presence of alkaloids, flavonoids, and phenolics. Niosomes were prepared by the thin-film hydration method using varying ratios of cholesterol and surfactants, followed by optimization based on key formulation parameters. The optimized formulation exhibited favorable physicochemical properties, including uniform dispersibility, an average particle size of 164.03 nm, a zeta potential of −27.7 mV, and high entrapment efficiency. SEM analysis revealed spherical vesicles with smooth surfaces. Antimicrobial efficacy was assessed against selected Gram-positive and Gram-negative bacterial strains. In vitro antibacterial evaluation using the well diffusion assay demonstrated superior activity of the niosomal extract compared to the plain extract, with inhibition zones of 14 mm against *Staphylococcus aureus* and 9 mm against *Escherichia coli*. Stability studies conducted over 30 days confirmed consistent particle size, morphology, and antibacterial activity under refrigerated conditions. Overall, the findings establish the niosomal system as a promising delivery vehicle for enhancing the stability and antimicrobial efficacy of plant-based bioactives.

KEYWORDS: *Aquilegia fragrans*; niosomes; lipid-based vesicles; antibacterial activity; phytochemicals; stability.

1. INTRODUCTION

The ideal drug delivery system delivers drug at rate dictated by the need of the body over the period of treatment and it channels the active entity solely to the site of action. Drug targeting can be defined as the ability to direct a therapeutic agent specifically to desired site of action with little or no interaction with non-target tissue **(Sengar, 2023).** Paul Ehrlich, in 1909, initiated the era of development for targeted delivery when he envisaged a drug delivery mechanism that would target directly to diseased cell. Since then, numbers of carriers were utilized to carry drug at the target organ/tissue, which include immunoglobulins, serum proteins, synthetic polymers, liposomes, microspheres, erythrocytes, niosomes etc **(Keshav, 2015).** Advantage of niosomes is the development of a simple practical method for the routine and large-scale production without the use of pharmaceutically unacceptable solvents. In recent years, niosomes have been extensively studied for their potential to serve as carriers for delivery of drugs, antigens, hormones and other bioactive agents **(Pirojiya and Dudhat 2025).**

Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkylpolyglycerol ether class and cholesterol with subsequent hydration in aqueous media **(More, 2018).** Niosomes are promising

vehicle for drug delivery and being non-ionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. The focus of this review is to bring out the various methods of preparation, characterizations, factors affecting, advantages and applications of niosomes. In niosomes, the vesicles forming amphiphile is a non-ionic surfactant such as Span – 60 which is usually stabilized by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate **(Patel *et al.*, 2022).**

The applications of niosomes are wide-ranging, from drug delivery and gene therapy to the release of cosmetic agents and antioxidants. Their ability to encapsulate sensitive compounds, protect them from premature degradation, and efficiently deliver them to the target site makes them essential tools for personalized medicine and the development of advanced therapies **(Mitchell *et al.*, 2021).** However, niosome design must be adjusted according to the characteristics of the molecule to be transported and the specific needs of the application. Niosomes represent an innovative solution to overcome bioavailability limitations and bioactive molecule stability. Their structural and functional versatility, combined with an optimized design, positions them as one of the most promising strategies in the development of controlled release systems, with applications in scientific and technological fields **(Pakulska *et al.*, 2016).**

Medicinal plants have long served as a valuable source of therapeutic agents due to their diverse phytochemical constituents and broad pharmacological potential. Among these, *Aquilegia fragrans*, a member of the Ranunculaceae family, has attracted attention for its traditional applications in managing various ailments. The plant is known to contain a range of bioactive compounds, including flavonoids, alkaloids, and phenolic derivatives, which are associated with antimicrobial, antioxidant, and anti-inflammatory activities **(Nardi, 2015).** Formulating plant extracts into stable dosage forms enhances their bioavailability, stability, and therapeutic potential. In this context, characterization of *Aquilegia fragrans* extract plays a crucial role in understanding its physicochemical properties and biological activity. Evaluating its antimicrobial effect against pathogenic microorganisms further highlights its potential application in developing safe and effective plant-based antimicrobial formulations **(McClements *et al.*, 2021).**

The present study aims to formulate and characterize niosomal vesicles containing *Aquilegia fragrans* extract and to evaluate their antimicrobial activity against pathogenic microorganisms.

2. MATERIAL AND METHODS

2.1 Chemicals

Glacial Acetic Acid, Nitroprusside, Sodium Hydroxide, and Ammonia were obtained from Merck, a reputable supplier of analytical reagents. Rankem provided the 1% Copper Sulphate Solution, and Clorofiltind provided the Chloroform, Conc. HCl and 95% Alcohol. Himedia provided the Magnesium. Molychem provided the Ethanol. Conc. H_2SO_4 were obtained from Fizmerck and Researchlab provided the Petroleum ether.

2.2 Collection of Plant Material

Aquilegia fragrans plant leaves were harvested, and they were dried for three days in the shade at room temperature. Dried plant components were kept in sealed glass containers in a dry, cool atmosphere to avoid contamination and deterioration. A plant taxonomist confirmed the identity and purity of the medicinal herb *Aquilegia fragrans.*

2.3 Process of Plant Material Extraction

The extraction of *Aquilegia fragrans* plant leaves using a continuous hot percolation method by Soxhlet apparatus with ethanol and petroleum ether was carried out following a standardized protocol. The plant leaves were first dried in the shade and grind into a fine powder. A weighed amount (300g) of the powdered material was placed inside a porous thimble and loaded into the Soxhlet extractor. Petroleum ether was used first at 60°C for non- polar compound extraction, followed by ethanol for polar compounds. The round-bottom flask was filled with 300–500 mL of the respective solvent and heated using a water bath. As the solvent evaporated, it condensed in the reflux condenser and repeatedly percolated through the plant material, dissolving the bioactive compounds. The extraction process was continued for 6–8 hours until the solvent in the siphon tube became colorless. After extraction, the solvent was removed using a rotary evaporator, and the concentrated extract was further dried under at 40°C in a rotary vacuum evaporator (Buchi type). The dried extract was weighed, and extract's % yield was calculated using the following formula:

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

The prepared dried extract was then stored at low temperatures for further phytochemical analysis and organoleptic characters (percentage yield, colour and odour) **(Khandelwal, 2008).**

2.4 Preliminary phytochemical investigations

Preliminary phytochemical investigations is the systematic study of the chemical substances found in plants called phytochemicals, which are responsible for their medicinal, nutritious, or poisonous qualities. These investigations were carried out to check and identify the effective constituents such as alkaloids, flavonoids, tannins, saponins, and phenolics of leaves extract *Aquilegia fragrans*. Investigated for as per the standard procedure reported practical pharmacognosy books **(Kokate *et al.*, 2001).**

2.5 The Organoleptic Studies of *Aquilegia fragrans*

Visual observation was used to assess organoleptic

qualities. Organoleptic studies of *Aquilegia fragrans*, such as general appearance, color, odor, and condition, were conducted and observed **(Semuli, 2014).**

2.6 Solubility study of *Aquilegia fragrans*

The qualitative solubility of *Aquilegia fragrans* in various solvents was investigated using Indian pharmacopoeia. *Aquilegia fragrans* was weighed and placed into a 10 ml test tube, where it was dissolved in the appropriate solvents (1 ml each of ethanol, DCM, distilled water, chloroform, and acetone) **(Jan *et al.*, 2021).**

2.7 Preparation of Lipid Based Vesicle System (Niosomes)

The film hydration method was developed among various methods for the formulation of niosomes. The following cholesterol and surfactant ratios were chosen: (100 to 500 mg) In chloroform containing 500 mg of extract, selected ratios of cholesterol and surfactants such as Span 60 were dissolved individually. A rotary flash evaporator was used to evaporate the solvent at a reduced pressure and a maintained temperature between 50℃ until a fragile lipid film was formed. At 50°C, the flask was continued rotating at 50°C for 1 hour, while the adhered film inside the round bottom flask was rehydrated with 30 ml of Phosphate buffer saline (PBS) pH 7.4 for 1 hour. After collecting the hydrated noisome, it was placed into a beaker and sonicated using bath sonicator for 20 mins to get niosomal dispersion. The sonicated dispersion was kept aside without disturbing the system at room temperature (RT) for swelling of vesicles, and then it was stored for 12 hours at 2-5°C **(Hegdekar et al., 2023, Rajak et al., 2022).**

Table 1: Composition of Niosomes formulation.

Formulations	Cholesterol (mg)	Span 60 (mg)	Chloroform (ml)	Extract (mg)	Phosphate buffer pH 7.4	Temperature (°C)
NSV 1	500	100	20 ml	300	30	50°C
NSV 2	400	200	20 ml	300	30	50°C
NSV 3	300	300	20 ml	300	30	50°C
NSV 4	200	400	20 ml	300	30	50°C
NSV 5	100	500	20 ml	300	30	50°C

2.8 Characterization of Niosomes

2.8.1 Particle size

One of the most crucial parameters for characterizing a Niosomes is its size. The Malvern Zeta sizer (Malvern Instruments) was used to measure the Niosomes's size (Jain et al., 2011).

2.8.2 Zeta potential

To find the particle charge and the velocity at which the particles moved in an electric field, the zeta potential was measured. Zetasizer Malvern instruments were used to evaluate the Niosomes (Balla et al., 2020).

2.8.3 Scanning Electron Microscopic (SEM)

The optimized Niosomes's morphological characteristics were obtained by employing the electron beam from a scanning electron microscope. A sputter coater in vacuum was then used to coat the Niosomess with a thin coating (2–20 nm) of metal, such as platinum, palladium, or gold. After that, the pretreatment specimen was struck by an electron beam, producing secondary electrons called augers. Rutherford and Kramer's Law was used to select and process just the electrons scattered at a 90° angle from this interaction between the electron beam and the specimen's atoms in order to produce surface topography photographs (**Ahmed *et al.*, 2020**).

2.9 Anti-microbial activity of Niosomes by Well diffusion assay

- **Preparation of Nutrient Agar Media**

In 100 milliliters of purified water, 2.8 grams of Nutrient Media were dissolved. The media's pH was measured prior to sterilization. The media was autoclaved for 15 minutes at 121˚C and 15 pounds of pressure to sterilize it. After nutrient media poured into plates, the plates were put in a laminar air flow until the agar solidified.

- **Well Diffusion Assay**

Standardized to 108 CFU/ml of bacteria, *E. coli* and *S.aureus* bacterial suspension were placed in the shaker. After that, 100µl of the broth's inoculums (108 CFU/ml) were extracted using a micropipette and inoculated onto a fresh, sterile, solidified Agar Media Plate. The entire sterile agar surface was covered with the inoculums using a sterile spreader to inoculate the agar plate. Using a sterile cork-borer, four 6- mm wells were borde into the solidified Agar Media Plate. The niosomes and extract (1 and 1.5 mg/ml) solution was then prepared for inoculation into the wells. A sample of 100 µl was loaded (**Mohammadi-Sichani *et al.*, 2012**).

- **Incubation period for observe zone of inhibition on agar plates**

Before being incubated for 18 to 24 hours at 37°C, the inoculated Agar Media Plate was let to diffuse for roughly 30 minutes at roomtemperature. After incubation, plates were checked to see if a clear zone formed around the well, indicating that the tested formulation had antibacterial activity. Millimeters of the zone of inhibition (ZOI) were measured and analyzed. Using a ruler held on the back of the inverted Petri plate, zones were measured to the nearest millimeter. A dark, non- reflective background was placed a few inches above the Petri plate. The diameters of the well and the zone of total inhibition (as perceived by

the naked eye) were measured (**Manandhar *et al.*, 2019).**

2.10 Stability study

The stability of the Niosomes formulation was examined for three months at accelerated temperatures of 25 0C±2 0C and 60 ± 5% RH and 40 0C±2 0C and 70 ± 5% RH. Physical attributes of the formulation, including color, order, appearance, and particle size, were evaluated at 30, 45, 60, and 90 days (3 months) (Khan et al., 2020).

3. RESULTS

3.1 Plant collection Table 2: Plant collection

Plant name	Plant part used	Weight
Aquilegia fragrans	Leaves	300 gm

3.2 Percentage Yield

The percentage yield is particularly important in phytochemical extraction since it helps identify the standard extraction efficiency for a particular plant, different parts of the same plant, or different solvents.

Table 3: Percentage Yield of crude extracts of *Aquilegia fragrans* extract

Plant name	Solvent	Color of extract	Theoretical weight	Yield(gm)	% yield
Aquilegia fragrans	Pet ether	Greenish	300	4.51	0.79%
	Ethanol	Green	278	5.43	2.63%

3.3 Preliminary Phytochemical study Table 4: Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Ethanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Present
1.2	Mayer's reagent test	Absent	Present
1.3	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Present	Present
2.2	Killer-Killiani test	Present	Present
Carbohydrates			
3.1	Molish's test	Present	Present
3.2	Fehling's test	Present	Present
3.3	Benedict's test	Present	Present
3.4	Barfoed's test	Absent	Present
3.5	Iodine Test	Absent	Present
Flavonoids			
4.1	Shinoda Test	Absent	Present
Tannin and Phenolic Compounds			
5.1	Ferric Chloride test	Present	Present
5.2	Lead Acetate Test	Absent	Present
5.3	Gelatin Test	Absent	Present
Saponin			
6.1	Foam test	Present	Present
6.2	Froth Test	Present	Present
Test for Triterpenoids and Steroids			
7.1	Salkowski's test	Present	Absent
7.2	Libbermann-Burchard's test	Present	Absent

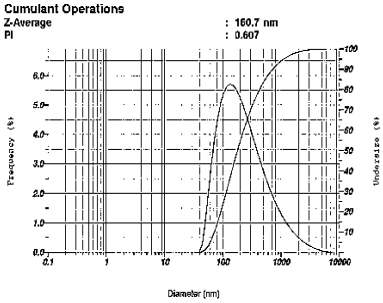
3.4 Organoleptic properties

Table 5: The Organoleptic Studies of *Aquilegia fragrans* leaves extract.

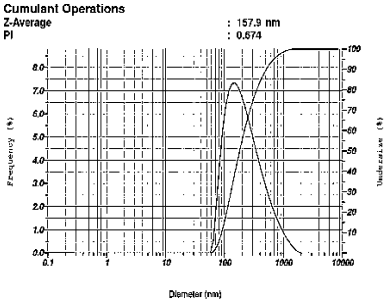
Aquilegia fragrans	**Study**
Colour	Greenish
Odour	Musky
Appearance	Greenish brown

3.5 Evaluation parameter of nonionic surfactant vesicle formulation

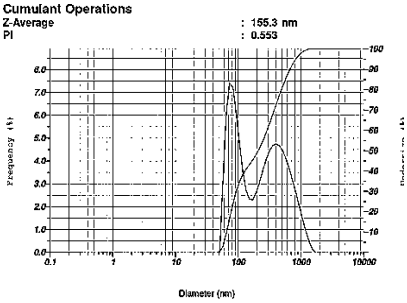
3.5.1 Particle Size



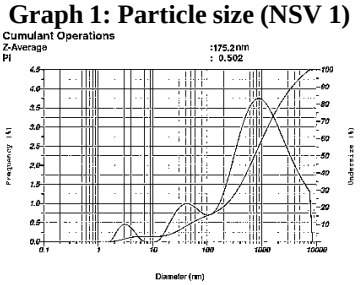
Graph 1: Particle size (NSV 1)



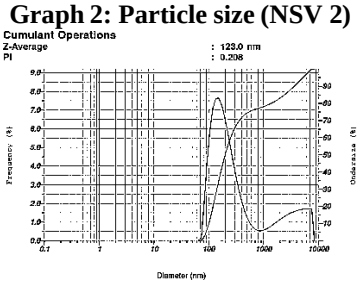
Graph 2: Particle size (NSV 2)



Graph 3: Particle size (NSV 3)



Graph 4: Particle size (NSV 4)

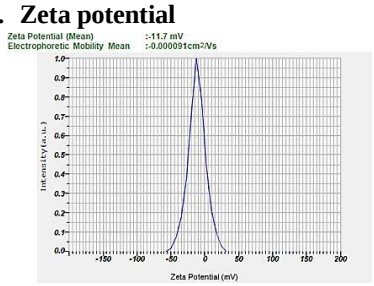


Graph 5: Particle size (NSV 5)

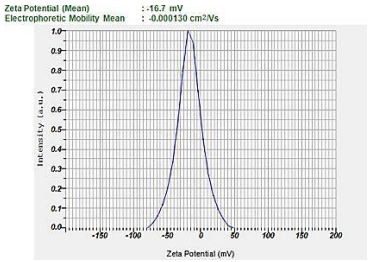
Table 6: Particle size.

Formulation code	Particle size (nm)	PI Value
Niosomes NSV 1	160.7 nm	0.607
Niosomes NSV 2	157.9 nm	0.674
Niosomes NSV 3	155.3 nm	0.553
Niosomes NSV 4	175.2 nm	0.502
Niosomes NSV 5	**123.0 nm**	**0.208**

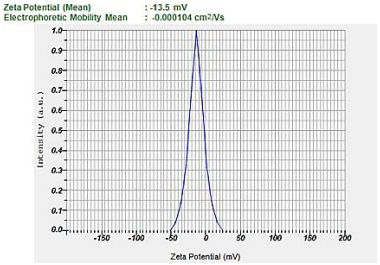
3.5.2. Zeta potential



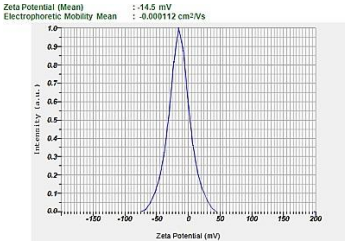
Graph 6: Zeta potential (NSV 1)



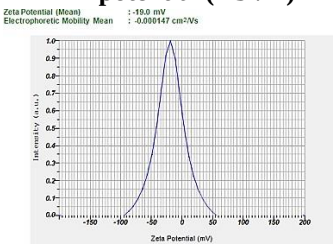
Graph 7: Zeta potential (NSV 2)



Graph 8: Zeta potential (NSV 3)



Graph 9: Zeta potential (NSV 4)



Graph 10: Zeta potential (NSV 5)

Table 7: Zeta potential.

Formulation Code	Zeta potential
Niosomes NSV 1	-11.7 mV
Niosomes NSV 2	-16.7 mV
Niosomes NSV 3	**-13.5 mV**
Niosomes NSV 4	-14.5 mV
Niosomes **NSV 5**	-19.0 mV

3.5.2.1 Scanning Electron Microscopic (SEM)

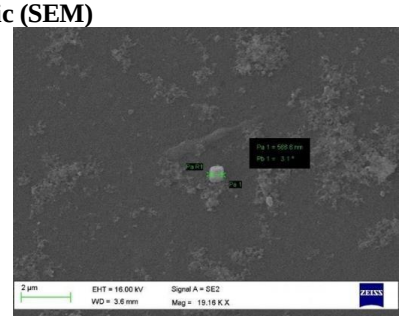
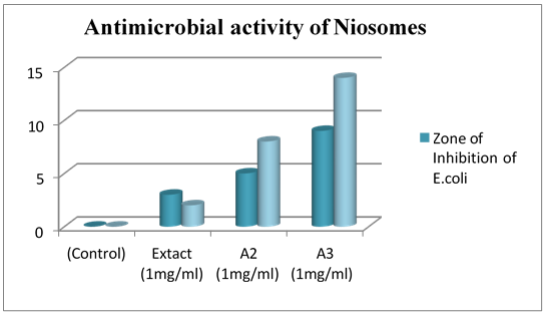


Figure 1: SEM (F5).

3.6 Results of antimicrobial activity of Niosomes F5 formulation

Table 8: Antimicrobial activity of Niosomes *against E.coli and S. aureus.*

Sample Name (mg/ml)	Zone of Inhibition (mm) of *E. coli*	Zone of Inhibition (mm) of *S. aureus*
D(Control)	0 mm	0 mm
C Extract (1mg/ml)	6 mm	5 mm
B (1mg/ml)	8 mm	7 mm
A (1.5mg/ml)	12 mm	11 mm



Graph 11: Graphical representation of antimicrobial activity of Niosomes *against E. coli and S. aureus.*

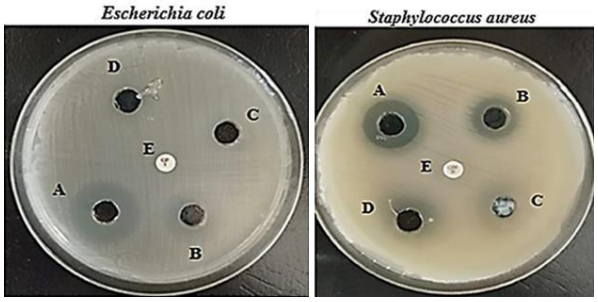


Figure 2: Antimicrobial activity against *E. coli.*

3.7 Stability study

Table 9: Stability Study of Niosomes (F5) formulation.

Time (Days)	$25^0C \pm 2\ ^0C$ and 60 ± 5% RH				$40^0C \pm 2\ ^0C$ and 70 ±5% RH			
	Colour	Odour	Appeanance	Particle size nm	Colour	Odour	Appea rance	Particle size nm
0	Yellowish milky	Musky	Liquid	164.03 nm	Greenish	Musky	Liquid	164.03nm
30	Yellowish milky	Musky	Liquid	164.11 nm	Greenish	Musky	Liquid	164.17nm
45	Yellowish milky	Musky	Liquid	164.10 nm	Greenish	Musky	Liquid	164.23nm
60	Yellowish milky	Musky	Liquid	164.28 nm	Greenish	Musky	Liquid	164.55 nm
90	Yellowish milky	Musky	Liquid	165.01 nm	Greenish	Musky	Liquid	164.53 nm

4. CONCLUSION

In conclusion, the niosomal formulation of *Aquilegia fragrans* extract was successfully developed and optimized based on key formulation and evaluation parameters. The digital results supported that the formulation achieved high percentage yield, confirmed phytochemical presence, and exhibited desirable physical properties, including optimal particle size, stable zeta potential, and consistent morphology. The antibacterial study highlighted a significant improvement in antimicrobial activity when the extract was encapsulated in niosomes, validating the potential of the vesicle system in enhancing the bioactivity of herbal extracts. Furthermore, the stability study confirmed the robustness of the formulation over time, establishing the niosomal system as an effective and stable delivery vehicle for plant-based antibacterial agents.

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