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Development And Charcterization of Oral Bioavailability Enhancing Formulation of Amaranthus Viridis Loaded Non-Ionic Surfactant Vesicle

Dipesh Gour[1], Dr. B.K. Arjariya[2], Praveen Bhawsar[3]
[1]*Research Scholar, Malhotra College of Pharmacyt*
[2]*Professor,HOD, Malhotra College of Pharmacy*
[3]*Associate Professor, Academic Incharge, Malhotra College of Pharmacy*

Abstract

The present study focuses on the development and characterization of an oral bioavailability-enhancing formulation of Amaranthus viridis using non-ionic surfactant vesicles (niosomes). Methanolic extraction of Amaranthus viridis leaves yielded a high concentration of bioactive phytoconstituents, including flavonoids, phenolics, alkaloids, and glycosides. The extract was successfully encapsulated into niosomes using the thin-film hydration method. The optimized formulation exhibited nanoscale particle size (~164 nm), high zeta potential (-38.6 mV), spherical morphology, and good physical stability. Quantitative analysis confirmed high phenolic and flavonoid content, contributing to antioxidant potential. The niosomal formulation showed enhanced antimicrobial activity against Escherichia coli and Staphylococcus aureus compared to the plain extract. Stability studies indicated no significant changes in physical properties over 90 days under accelerated conditions. Overall, the results suggest that niosomal delivery of Amaranthus viridis extract is a promising approach to improve oral bioavailability and therapeutic efficacy of plant-based formulations.

Keywords; Amaranthus Viridis, Non-Ionic Surfactant Vesicles (Niosomes), Bioactive Phytoconstituents, Medicinal Plants (MPs).

INTRODUCTION

Medicinal plants play a significant role in global healthcare systems due to their proven therapeutic potential, cultural acceptance, and comparatively fewer side effects than synthetic drugs. A substantial portion of the world's population depends on plant-based medicines for primary healthcare (Ali et al., 2021). These plants are rich sources of bioactive phytochemicals such as alkaloids, flavonoids, phenolics, glycosides, and saponins, which contribute to their pharmacological activities (Roy et al., 2025). The fact that many modern drugs are derived from plant sources emphasizes the importance of scientific evaluation of medicinal plants to validate traditional knowledge and support pharmaceutical development (Rehman et al., 2020).

The therapeutic effectiveness of herbal drugs largely depends on the delivery system used. Conventional dosage forms often exhibit limitations such as poor solubility, instability, low permeability, and reduced bioavailability, which restrict their clinical performance. To address these challenges, novel drug delivery systems (NDDS) have been developed to enhance drug stability, improve bioavailability, minimize side effects, and provide controlled and sustained drug release. NDDS integrates principles from pharmaceutics, polymer science, and molecular biology to improve drug targeting, particularly for complex herbal formulations containing multiple active constituents (Seema 2014).

Niosomes, which are vesicular systems formed from non-ionic surfactants and cholesterol, have emerged as an effective nanocarrier in NDDS. They are capable of encapsulating hydrophilic, lipophilic, and amphiphilic drugs within stable bilayer structures (Chandu et al., 2012). Compared to liposomes, niosomes offer advantages such as improved stability, lower toxicity, higher drug entrapment efficiency, and enhanced membrane permeability. Their properties can be modified by adjusting surfactant type, cholesterol concentration, vesicle size, and surface charge, enabling controlled and targeted drug delivery (Moammeri et al., 2023).

The structural characteristics of niosomes depend on factors such as surfactant hydrophilic–lipophilic balance (HLB), preparation technique, and lipid composition. Various niosomal systems, including multilamellar, unilamellar, proniosomes, and deformable niosomes, have been developed to meet different therapeutic and administration requirements (Arunachalam et al., 2012).

Enhancing oral bioavailability remains a major challenge for herbal drugs due to poor solubility and permeability (Verma et al., 2011). Niosomes effectively address this issue by improving dissolution, protecting drugs from gastrointestinal degradation, reducing first-pass metabolism, and enabling sustained drug release (Bácskay et al., 2025).

Amaranthus viridis is a medicinal plant widely recognized for its nutritional and therapeutic properties, including anti-inflammatory, antioxidant, antimicrobial, antidiabetic, hepatoprotective, and wound-healing activities. Incorporating its extract into a niosomal delivery system offers a promising strategy to enhance bioavailability, stability, and therapeutic efficacy, thereby integrating traditional herbal medicine with modern nanotechnology for improved clinical outcomes (Liga et al., 2024).

PLANT PROFILE

Figure 1: Amaranthus Viridis

Habitat of Amaranthus Viridis

Amaranthus Viridis, or green amaranth, thrives in tropical and subtropical regions, particularly in disturbed habitats like agricultural fields, roadsides, and wastelands. It prefers loamy, well-drained soils and can tolerate both moist and dry conditions, though it grows best with moderate moisture. This sun-loving plant is commonly found in areas with full sunlight but can also tolerate partial shade. It is often considered a weed in crops but is also used as an edible vegetable and fodder in various parts of the world. It adapts well too low to medium elevations, often near water sources like rivers or irrigation channels (Fouad et al., 2025).

Botanical Description of A. viridis

Amaranthus Viridis is an annual herb with an upright, light green stem that grows to about 60–80 cm in height. Numerous branches emerge from the base, and the leaves are ovate, 3–6 cm long, 2–4 cm wide, with long petioles of about 5 cm. The plant has terminal panicles with few branches, and small green flowers with 3 stamens (KurbonovicH, 2017).

Medicinal importance of Amaranthus Viridis whole plant

Amaranthus Viridis, or green amaranth, has significant medicinal importance in traditional medicine, particularly in tropical and subtropical regions. It is recognized for its anti-inflammatory, antimicrobial, and antioxidant properties. The plant is commonly used to reduce inflammation, especially in conditions like arthritis, and its antioxidant compounds help combat oxidative stress, potentially preventing chronic diseases (Haider et al., 2023). Amaranthus Viridis also has antimicrobial effects, making it useful for treating infections, wounds, and skin conditions. Additionally, it acts as a natural diuretic, promoting urination and aiding in the treatment of fluid retention and urinary tract infections. The plant is also believed to support liver health and may assist in detoxification. Its leaves and seeds are rich in essential nutrients, offering both medicinal and nutritional benefits, including supporting general well-being. While these uses are well-documented in traditional practices, more scientific research is needed to fully validate its medicinal potential (Jaafar & Kadhim, 2025).

Pharmacological Activity of Amaranthus Viridis

Amaranthus Viridis has demonstrated significant anti-inflammatory effects in various studies. The plant's leaf and stem extracts contain flavonoids, saponins, and other bioactive compounds that can reduce inflammation. This activity makes it useful in managing inflammatory conditions like arthritis and other inflammatory diseases (Ji et al., 2022). The plant is rich in antioxidants, particularly

flavonoids, polyphenols, and vitamins like Vitamin C. These compounds help neutralize free radicals in the body, reducing oxidative stress. As a result, Amaranthus Viridis is considered beneficial for preventing oxidative damage associated with chronic conditions like cancer, cardiovascular diseases, and aging (Ji et al., 2022).

Amaranthus Viridis has been shown to possess antimicrobial and antifungal properties. Its extracts have demonstrated activity against various bacterial strains, including both Gram-positive and Gram-negative bacteria, as well as fungal pathogens. This makes it useful in treating infections, particularly skin infections, and wounds (Haider et al., 2023). Preliminary studies suggest that Amaranthus Viridis may help regulate blood sugar levels. The plant's hypoglycemic potential has been observed in animal models, and it is believed to improve insulin sensitivity and reduce blood glucose levels. While further research is needed to confirm these findings, it holds promise as an adjunct in managing diabetes (Ji et al., 2022).

Traditionally, Amaranthus Viridis has been used as a natural diuretic. The plant helps promote urination, which can assist in reducing fluid retention, managing edema, and supporting kidney function. This diuretic effect also aids in the elimination of toxins from the body (Ji et al., 2022). Amaranthus Viridis is believed to have hepatoprotective properties, supporting liver function and protecting it from damage. Some studies suggest that the plant's compounds may enhance liver enzyme activity and promote detoxification, potentially making it beneficial in conditions like liver diseases or jaundice (Haider et al., 2023).

Extracts of Amaranthus Viridis have been reported to exhibit mild analgesic (pain-relieving) and antipyretic (fever-reducing) effects. These properties make it useful in managing mild to moderate pain and fever (Kumar et al., 2023). The plant has shown promise in promoting wound healing due to its antimicrobial, anti-inflammatory, and tissue-regenerating properties. It is traditionally applied topically to cuts and wounds to speed up healing and reduce the risk of infection (Moammeri et al., 2023).

MATERIAL AND METHODS

In research, reagents are specialized chemicals employed to initiate or monitor particular reactions, often serving as catalysts or indicators. Chemicals supply the essential materials needed for experiments, including solvents, reactants, and compounds used for analysis or synthesis. Instruments like spectrometers, chromatographs, and microscopes play a vital role in precisely measuring, analyzing, and observing experimental results.

Table 1: List of reagent chemical, glassware's, and instruments

S. No	**Reagents and chemicals**	**Company Name**
1.	Sodium Hydroxide	Merk
2.	Chloroform	Clorofilt ind.
3.	Soya-Lacithin	Lobagens
4.	Ethanol	Molychem
5.	Glacial Acetic Acid	Merck
6.	Nitroprusside	Merck
7.	Cholesterol	NR chem
8.	Conc. HCl	Clorofiltind
9.	95% Alcohol	Clorofiltind
10.	% Copper Sulphate Solution	Rankem
11.	Petroleum ether	Researchlab
12.	Magnesium 1	Himedia
13.	Conc. H_2SO_4	Fizmerck
14.	Ammonia	Merk
S. No	**List of Glassware's**	**Type/Company**
1.	Volumetric flask	Borocil
2.	glass slide	Borocil
3.	Measuring cylinder	Borocil
4.	Conical flask	Borocil
5.	Beaker	Borocil
6.	Glass rod	Borocil
7.	Petri dishes	Borocil
8.	Test tubes	Borocil
S. No	**Instruments**	**Model**
1.	Weighing Machine	Sirtech
2.	Magnetic stirring	Remi
3.	Boiling water bath	Universal
4.	Melting point apparatus (Tempo)	Tempo
5.	Vortex shaker	Scientech (SE-146)
6.	UV-Visible Spectrophotometer	Shimadzu 1700
7.	Zeta sizer	Malvern Instruments
8.	Lyophilizer	Laborax
9.	Hot air oven	Scientech
10.	Brookfield viscometer ic	Brookfield DV-II+Pro (LV)
11.	Digital pH meter	Ei
12.	Scanning Electron Microscop	Hitachi High-Tech
13.	dialysis bag	Aldrich
14.	Desiccator	Polycab

Collection and Taxonomic Authentication of Plant Material

- **Plant Collection:** About 245 grams of Amaranthus Viridis were gathered from a specific locality in Bhopal.
- **Authentication:** The plant specimen was identified and verified by a qualified botanist for accurate taxonomic confirmation.
- **Initial Cleaning:** The harvested leaves were carefully washed to remove dust and other impurities.
- **Drying Process:** The plant material was dried naturally in the shade at room temperature over several days to reduce moisture content.
- **Pulverization:** After complete drying, the plant part was ground into a fine powder.
- **Storage:** The powdered material was kept in clean, airtight containers to preserve its quality and prevent contamination.

Preparation and Extraction of Plant Sample Using the Soxhlet Method

- **Purpose:** The Soxhlet extraction technique is widely employed to efficiently isolate bioactive constituents from natural plant materials.
- **Sample Preparation:** A specific amount of dried Amaranthus Viridis powder was placed inside a cellulose thimble for extraction.
- **Setup:** The thimble was inserted into the main chamber of the Soxhlet extractor, which was attached to a round-bottom flask containing a solvent mixture of methanol and petroleum ether.
- **Working Principle:**
  - When heated, the solvent vaporizes and subsequently condenses, dripping onto the plant material within the extraction chamber.
  - Upon filling to the siphon point, the solvent containing dissolved compounds automatically siphons back into the boiling flask.
  - This cycle of evaporation, condensation, and siphoning repeats multiple times, ensuring thorough extraction.
- **Outcome:** This continuous reflux process facilitates the effective extraction of soluble phytochemicals from the plant material into the solvent mixture.
- **Reference: (Adapted from López et al., 2020)**

Figure 2: Soxhlation process of plant extract by methanol & petroleum ether

Determination of Extract Yield

Following the completion of the Soxhlet extraction, the solvent carrying the extracted compounds was concentrated by removing the solvent under reduced pressure. The resulting crude extract was then weighed accurately using an analytical balance. The extraction yield was determined as a percentage relative to the initial dry weight of the plant material, calculated using the following formula:

$$Extract\ Yield\ (\%) = \left(\frac{Weight\ of\ Extract}{Initial\ Dry\ Weight\ of\ Plant\ material}\right) \times 100$$

Investigation of Bioactive Plant Constituents of Amaranthus Viridis (Shaikh and Patil 2020)

The phytochemical screening of Amaranthus viridis extract was carried out to identify the presence of major bioactive constituents using standard qualitative chemical tests.

(i) **Tests for Alkaloids:** Alkaloids were detected using Dragendorff's, Wagner's, Mayer's, and Hager's tests, where the formation of characteristic colored precipitates confirmed their presence.

(ii) **Test for Carbohydrates:** Carbohydrates were identified through Molisch's test, which produced a violet ring, and reducing sugars were confirmed

using Fehling's, Benedict's, and Barfoed's tests based on the formation of colored precipitates.

(iii) **Test for Saponins:** The presence of saponins was confirmed by the froth test, indicated by the formation of stable foam after shaking the extract with water.

(iv) **Test for Triterpenoids and Steroids:** Triterpenoids and steroids were identified using Liebermann–Burchard and Salkowski tests, which produced characteristic color changes such as bluish-green and reddish hues.

(v) **Test for Tannin and Phenolic Compounds:** Tannins and phenolic compounds were detected using ferric chloride, gelatin, and lead acetate tests, evidenced by the formation of blue coloration or white precipitates.

(vi) **Test for protein and amino acids:** Proteins and amino acids were confirmed by Biuret's and ninhydrin tests through the appearance of violet, pink, or blue coloration.

(vii) **Test for Flavonoids:** The presence of flavonoids was identified using lead acetate and alkaline reagent tests, indicated by yellow coloration or precipitate formation.

(viii) **Test for Glycosides:** Glycosides were detected using Borntrager's and Keller–Killiani tests, confirming the presence of anthraquinone and cardiac glycosides through characteristic color changes.

Overall, the phytochemical investigation confirmed that Amaranthus viridis contains a wide range of bioactive constituents, supporting its traditional medicinal uses and potential for pharmaceutical applications.

Quantitative Estimation of Phytoconstituents

The quantitative analysis of phytoconstituents was performed to determine the concentrations of key bioactive compounds present in the methanolic extract of Amaranthus Viridis. Standard spectrophotometric methods were employed, utilizing calibration curves constructed with known standards for each phytochemical class.

(a). Total phenolic Content (TPC) estimation (Sánchez et al., 2013): Method preparation

(i) Prepare a methanolic extract of Amaranthus Viridis at a concentration of 1 mg/mL.

(ii) Transfer 40 µL of this extract into a clean test tube.

(iii) Add 3.16 mL of distilled water to the test tube containing the extract.

(iv) Introduce 200 µL of Folin-Ciocalteu reagent to the mixture.

(v) Mix gently and allow the reaction to proceed by incubating the mixture at room temperature for 8 minutes.

(vi) Add 600 µL of 7.5% sodium carbonate solution and mix the contents thoroughly.

(vii) Incubate the mixture at 40°C for 30 minutes to facilitate color development.

(viii) Measure the absorbance of the blue-coloured solution at 760 nm using a spectrophotometer.

(ix) Construct a standard calibration curve using gallic acid solutions with known concentrations of 30, 50, 70, 90, and 110 µg/mL.

(x) Determine the total phenolic content of the extract by comparing its absorbance to the calibration curve, and express the result as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

(b). Total flavonoid content (TFC) estimation: Method preparation

(i) Accurately weigh 0.1 g of the Amaranthus Viridis plant extract.

(ii) Dissolve this sample in 1 mL of deionized water to prepare the stock solution.

(iii) Transfer 0.5 mL of the prepared solution into a clean test tube.

(iv) Add 1.5 mL of 95% ethanol to the test tube containing the sample.

(v) Introduce 0.1 mL of 10% aluminum chloride hexahydrate ($AlCl_3$) solution to the mixture.

(vi) Add 0.1 mL of 1 M potassium acetate (CH_3COOK) solution.

(vii) Pour 2.8 mL of deionized water into the test tube to complete the volume.

(viii) Mix the solution thoroughly to ensure homogeneity.

(ix) Allow the reaction mixture to incubate at room temperature for 40 minutes to enable complex formation.

(x) Measure the absorbance of the resulting solution at 510 nm using a spectrophotometer, with deionized water serving as the blank.

(xi) Prepare a standard calibration curve using rutin solutions at concentrations of 30, 50, 70, 90, and 110 µg/mL.

(xii) Determine the total flavonoid content by comparing the sample's absorbance against the rutin calibration curve.

(xiii) Express the flavonoid content as milligrams of rutin equivalents per gram of dry extract (mg RE/g) (Ghafar et al., 2017).

The Organoleptic Studies of Amaranthus Viridis

Organoleptic evaluation of Amaranthus Viridis was carried out using visual and sensory observations. Parameters such as appearance, color, odor, taste, and texture were assessed by a group of panelists to determine the quality and acceptability of the samples.

Solubility study of Amaranthus Viridis

The qualitative solubility of Amaranthus Viridis was examined in different solvents following the guidelines of the Indian Pharmacopoeia. A measured amount of the plant material was placed into a 10 ml test tube and dissolved separately in 1 ml of various solvents, including methanol, dichloromethane (DCM), distilled water, chloroform, and acetone.

Preparation Niosome formulation

Among the various techniques available, the film hydration method was employed for the formulation of niosomes. Specific ratios of cholesterol (50–250 mg) and Span 60 (a non-ionic surfactant) were selected and individually dissolved in chloroform containing 400 mg of the plant extract. The mixture was subjected to solvent evaporation using a rotary flash evaporator under reduced pressure at a controlled temperature of 50–55°C, leading to the formation of a thin lipid film on the inner wall of the round-bottom flask.

The flask was continuously rotated at the same temperature for 1 hour to ensure complete formation of the lipid layer. Following this, the dried film was hydrated with 20 ml of phosphate buffer saline (PBS, pH 7.4) while maintaining the temperature at 50–55°C for another 1 hour.

The resulting hydrated niosomal suspension was transferred to a beaker and sonicated in a bath sonicator for 20 minutes to obtain a uniform dispersion. After sonication, the dispersion was left undisturbed at room temperature to allow for the swelling and maturation of vesicles, and then it was stored at 2–5°C for 12 hours for stabilization (Chaw et al., 2013).

Table 2: Composition of Niosomes formulation

S. No	Formulations	Cholesterol (mg)	Span 60 (mg)	Chloroform (ml)	Extract (mg)	Phosphate buffer pH 7.4	Temperature (50^0C-55°C)
1	NSF 1	50	250	15 ml	400	23	500C-55°C
2	NSF 2	100	200	15 ml	400	23	500C-55°C
3	NSF 3	150	150	15 ml	400	23	500C-55°C
4	NSF 4	200	100	15 ml	400	23	500C-55°C
5	NSF 5	250	50	15 ml	400	23	500C-55°C

Characterization parameters of Niosomes:

Particle size

- Niosomal particle size was measured using a Zeta sizer (Malvern Instruments, UK).
- The technique used was dynamic light scattering (DLS).
- Millipore-filtered water was used to dilute the niosomal dispersion to the required scattering intensity.
- The diluted sample was transferred to a disposable cuvette for measurement.
- Measurements were performed at a constant temperature of 25°C (Gee et al., 2002).

Zeta potential

- Zeta potential was measured to assess the surface charge of the niosomal particles.
- The analysis was performed using a Zetasizer (Malvern Instruments, UK).
- Samples were diluted with Millipore-filtered water before measurement.
- The diluted dispersion was placed into a disposable folded capillary cell provided by the manufacturer.
- Measurements were conducted at a controlled temperature of 25°C.
- The obtained zeta potential values indicate the degree of electrostatic repulsion between particles (Bhattacharjee, 2016).

Scanning Electron Microscopic (SEM)

- The morphology of the optimized niosomes was examined using Scanning Electron Microscopy (SEM).
- Prior to imaging, the niosomal sample was coated with a thin metallic layer (2–20 nm) of gold, platinum, or palladium using a sputter coater under vacuum.
- The coated sample was then exposed to an electron beam in the SEM chamber.

- Interaction between the electron beam and the sample surface generated secondary electrons.
- These scattered electrons, particularly those deflected at 90° angles, were selected based on Rutherford and Kramer's Law to form detailed surface topography images.
- SEM images were used to study the shape, size, and surface characteristics of the niosomes (Mohammed and Abdullah 2018).

Anti-microbial activity of Niosomes by Well diffusion assay

Preparation of Nutrient Agar Media

A total of 2.8 grams of nutrient media was accurately dissolved in 100 milliliters of purified water. The pH of the solution was recorded before the sterilization process. The prepared medium was then autoclaved at 121°C under 15 psi pressure for 15 minutes to ensure complete sterilization. Once sterilized, the medium was poured into sterile Petri plates under aseptic conditions, and the plates were then placed inside a laminar airflow chamber to allow the agar to solidify.

Well Diffusion Assay

A bacterial suspension of Escherichia coli and Staphylococcus aureus was standardized to a concentration of 10^8 CFU/ml and incubated in a shaker. From this, 100 µl of the inoculum was carefully drawn using a micropipette and spread uniformly over the surface of a sterile, pre-solidified nutrient agar plate. A sterile glass spreader was used to evenly distribute the bacterial culture across the entire plate surface. Using a sterile cork borer, four wells (6 mm in diameter) were made in the agar. Solutions of the niosomal formulation and plant extract at concentrations of 1 mg/ml and 1.5 mg/ml were prepared. Then, 100 µl of each test sample was introduced into the wells. The plates were then incubated to assess antimicrobial activity (Srivastav and Das 2014).

Incubation period for observe zone of inhibition on agar plates

After adding the test samples into the wells, the agar plates were left undisturbed at room temperature for approximately 30 minutes to allow proper diffusion of the formulations into the medium. Following this, the plates were incubated at 37°C for 18 to 24 hours. Post-incubation, the plates were examined for the formation of clear zones around the wells, indicating antimicrobial activity. The zones of inhibition (ZOI) were measured in millimeters using a ruler placed on the back of the inverted Petri dish. For clarity and accuracy, a dark, matte background was positioned a few inches above the plate during measurement. Both the diameter of the wells and the total visible inhibition zone were recorded to the nearest millimeter by visual observation (Galvão et al., 2016).

Stability study

Study Design

Formulation was stored for 90 days under two accelerated conditions:

- 25 °C ± 2 °C with 60 % ± 5 % RH
- 40 °C ± 2 °C with 70 % ± 5 % RH

Sampling Time Points

Physical evaluations were performed at day 0, 30, 45, 60, and 90 (3 months).

Physical Parameters Assessed

- Color
- Odor
- General appearance
- Particle size

Protocol Alignment

Study conducted per ICH Q1A guidelines for accelerated stability testing, which specifies that niosomal dispersions under accelerated conditions should be monitored for changes in physical attributes over a 3- to 6-month period, typically at 40 °C / 75 % RH ± 5 % RH for accelerated studies and 25 °C / 60 % RH for long-term predictions

Outcome Comparison

- Observations from each time point (days 30, 45, 60, 90) were compared against baseline values at day 0.
- Any significant deviations in color, odor, appearance, or particle size were noted and analysed (Cha et al., 2011).

RESULTS AND DISCUSSION

Collection of plant material

Table 3: Collection of plant

S. No.	Plant name	Plant part used	Weight
1.	*Amaranthus Viridis*	Leaves	245

Determination of Extract Yield

The leaves of Amaranthus Viridis were collected and weighing 245 grams. Extraction was performed using two solvents: petroleum ether and methanol. The petroleum ether extract yielded a low percentage (0.85%) with a greenish-yellow color, while the methanol extract showed a much

higher yield of 6.20% with a dark green color. This indicates that methanol is a more efficient solvent for extracting phytochemicals from Amaranthus Viridis leaves.

Table 4: Percentage Yield of crude extracts of Amaranthus Viridis extract

S. No	Plant name	Solvent	Color of extract	Theoretical weight	Yield(gm)	% yield
1	*Amaranthus Viridis*	Pet ether	Greenish Yellow	245.6	2.10g	0.85%
2		Methanol	Dark green	240.67	14.93g	6.20%

Investigation of Bioactive Plant Constituents of Amaranthus Viridis

Table 4: Phytochemical test of Amaranthus Viridis extract of methanol

S. No.	Experiment	Presence or absence of phytochemical test
		Methanol extract
1.	**Alkaloids**	
1.1	Mayer's reagent test	Absent (- ve)
1.2	Wagner's reagent test	Present (+ ve)
1.3	Hager's reagent test	Present (+ ve)
1.4	Dragendroff's test	Present (+ ve)
2.	**Glycoside**	
2.1	Borntrager test	Present (+ ve)
2.2	Legal's test	Present (+ ve)
2.3	Killer-Killiani test	Present (+ ve)
3.	**Carbohydrates**	
3.1	Molish's test	Present (+ ve)
3.2	Fehling's test	Present (+ ve)
3.3	Benedict's test	Present (+ ve)
3.4	Barfoed's test	Absent (- ve)
4	**Flavonoids**	
4.1	Alkaline reagent test	Present (+ ve)
4.2	Lead Acetate test	Present (+ ve)
5	**Tannin and Phenolic Compounds**	
5.1	Ferric Chloride test	Present (+ ve)
5.2	Lead Acetate	Present (+ ve)
5.3	Gelatin test	Present (+ ve)
6.	**Saponin**	
6.1	Foam test	Present (+ ve)
7.	**Test for Triterpenoids and Steroids**	
7.1	Salkowski's test	Absent (-ve)
7.2	Libbermann-Burchard's test	Present (+ ve)
8.	**Test for Protein and Amino Acid**	
8.1	Biuret's Test	Present (+ ve)
8.2	Ninhydrin test	Absent (- ve)

The phytochemical screening of the methanol extract of Amaranthus Viridis leaves revealed the presence of several important bioactive compounds. Alkaloids were confirmed by positive results in Wagner's, Hager's, and Dragendorff's tests, although Mayer's test was negative. Glycosides were also present, as indicated by Borntrager, Legal's, and Killer-Killiani tests. Carbohydrates tested positive in Molish's, Fehling's, and Benedict's tests, while Barfoed's test was negative, suggesting the presence of complex carbohydrates rather than monosaccharides. Flavonoids and phenolic compounds, including tannins, were detected through multiple tests, indicating strong antioxidant potential. The presence of saponins was confirmed by the foam test. Among triterpenoids and steroids, only Liebermann-Burchard's test was positive, while Salkowski's test was negative, indicating selective steroid presence. Proteins were partially detected with a positive Biuret test but negative Ninhydrin test for amino acids.

Overall, the methanol extract contains a rich variety of phytochemicals that contribute to the therapeutic potential of Amaranthus Viridis.

Quantitative Estimation of Phytoconstituents

Table 5: Standard table for Gallic acid and Rutin

S. No.	Concentration (µg/ml)	Absorbance	
		Gallic acid	Rutin
1.	20	0.182	0.170
2.	40	0.220	0.195
3.	60	0.245	0.215
4.	80	0.274	0.235
5.	100	0.300	0.250

Table 6: Total Phenolic Content and total flavonoid content (TFC) in Amaranthus Viridis extract

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.179	86.8mg/gm
2	0.255	

3	0.300	
S. No	**Absorbance**	**TFC in mg/gm equivalent of Rutin**
1	0.169	42mg/gm
2	0.192	
3	0.225	

Total Phenolic content (TPC) estimation

The total phenolic content (TPC) of Amaranthus Viridis extract was determined using the Folin- Ciocalteu method with gallic acid as the standard. A standard calibration curve was prepared with gallic acid concentrations ranging from 20 to 100 µg/ml, showing a linear increase in absorbance values from 0.182 to 0. 300. Using this standard curve, the phenolic content of the extract was measured at different absorbance values. For instance, an absorbance of 0.179 corresponded to a TPC of 86.8 mg/g gallic acid equivalents. Higher absorbance values (0.255 and 0.300) indicate even greater phenolic content, confirming that Amaranthus Viridis leaves are rich in phenolic compounds. These phenolics are known for their antioxidant properties, suggesting potential health benefits of the extract.

Total Flavonoids content (TFC) estimation

The total flavonoid content (TFC) of Amaranthus Viridis extract was measured using a standard curve prepared with rutin as the reference compound. The rutin standard showed a linear relationship between concentration (20–100 µg/ml) and absorbance (0.170–0.250).

Based on this calibration, the extract's flavonoid content was quantified at different absorbance values. For example, an absorbance of 0.169 corresponded to a TFC of 42 mg/g rutin equivalents. Higher absorbance values (0.192 and 0.225) indicate increased flavonoid concentrations. These results confirm that Amaranthus Viridis leaves are a good source of flavonoids, which contribute to antioxidant activity and potential therapeutic effects.

Organoleptic properties

Table 7: The Organoleptic Studies of Amaranthus Viridis leaves extract

S. No	*Amaranthus Viridis*	Study
1	Colour	Greenish
2	Odour	Musky
3	Appearance	Greenish brown

The organoleptic properties of the Amaranthus Viridis leaves extract, such as color, odor, and appearance, were assessed. When analyzed, it was found that the plant extract had a greenish hue. According to studies, the extract has a musk-like smell and a semi-solid, greenish brown look. Result show in Table 8.

Table 8: Solubility study of Amaranthus Viridis

Drug	Solvents	Observation/Inference
Amaranthus Viridis	Water	Slightly soluble
	Ethanol	Freely soluble
	Methanol	Freely soluble
	Chloroform	Sparingly soluble
	DMSO	Freely soluble
	Acetone	Freely soluble

Characterization parameter of extract loaded Niosomes formulation

Physical properties

Table 9: Physical Appearance of Niosome formulation

S. No.	Formulation	Parameters	Observation
1.	Niosome formulation	Colour	Pale green
2.		Odour	herbal odour
3.		Appearance	semi-solid, uniform dispersion

The Niosome formulation exhibited a pale green colour, which reflects the natural hue of the Amaranthus Viridis extract, indicating that the extract was successfully loaded without degradation or significant alteration. The presence of a herbal odour further confirms that the characteristic scent of the plant extract was retained during formulation, suggesting minimal interference from the excipients used.

The formulation's semi-solid and uniform dispersion appearance demonstrates good physical stability and homogeneity, important factors for consistent dosing and ease of application. This consistency also suggests effective encapsulation and distribution of the extract within the niosomal vesicles. Overall, these physical properties indicate that the formulation is well- prepared, stable, and suitable for its intended use in delivering the herbal extract efficiently.

Particle size determination

Table 10: Particle size of Niosome formulation

S. No	Formulation	Particle size
1	Niosomes (NSF 1)	164.03nm
2	Niosomes (NSF 2)	166.79 nm
3	Niosomes (NSF 3)	246.2 nm
4	Niosomes (NSF 4)	178.93 nm
5	Niosomes (NSF 5)	251.3 nm

The particle size analysis of the Amaranthus Viridis-loaded niosomal formulations (NSF 1–NSF 5) revealed significant variation in size among the different batches. Particle size plays a crucial role in determining the efficiency, stability, and bioavailability of niosomal drug delivery systems. Among all the formulations, NSF 1 exhibited the smallest particle size of 164.03 nm, which falls within the ideal nanoscale range (100–200 nm) for enhanced drug penetration and cellular uptake. In contrast, formulations like NSF 3 (246.2 nm) and NSF 5 (251.3 nm) showed relatively larger particle sizes, which could potentially affect the formulation's performance. NSF 2 and NSF 4 were within acceptable limits but still larger than NSF 1. Therefore, based on particle size analysis, NSF 1 is considered the most effective formulation for further development due to its optimal nanosize and potential for improved therapeutic effectiveness.

Zeta potential determination

Table 11: Zeta potential of Niosome formulation

S. No	Formulation	Zeta potential
1	Niosomes (NSF 1)	-38.6 mV
2	Niosomes (NSF 2)	-27.7 mV
3	Niosomes (NSF 3)	-31.8 mV
4	Niosomes (NSF 4)	-33.0 mV
5	Niosomes (NSF 5)	-30.2mV

Zeta potential measurement is a critical parameter for assessing the stability of niosomal formulations. A higher magnitude of zeta potential, typically above ±30 mV, indicates better electrostatic repulsion between particles, which helps prevent aggregation and enhances formulation stability. Among the five formulations tested, NSF 1 showed the highest zeta potential of -38.6 mV, suggesting excellent colloidal stability. In comparison, NSF 2 had a lower value of -27.7 mV, which may result in reduced stability. NSF 3, NSF 4, and NSF 5 displayed moderate values ranging from -30.2 mV to -33.0 mV, which are acceptable but not as stable as NSF 1. Therefore, based on zeta potential analysis, NSF 1 is considered the most stable and promising formulation for further development.

Scanning Electron Microscopic (SEM)

The SEM image of the Niosome F1 formulation, captured at a magnification of 74,100x, shows smooth, spherical vesicles with a size scale around 200 nm. The vesicles appear uniform and well-dispersed, indicating good stability and proper niosome formation. The clear morphology confirms successful self-assembly of surfactant and cholesterol into stable nanosized vesicles suitable for drug delivery.

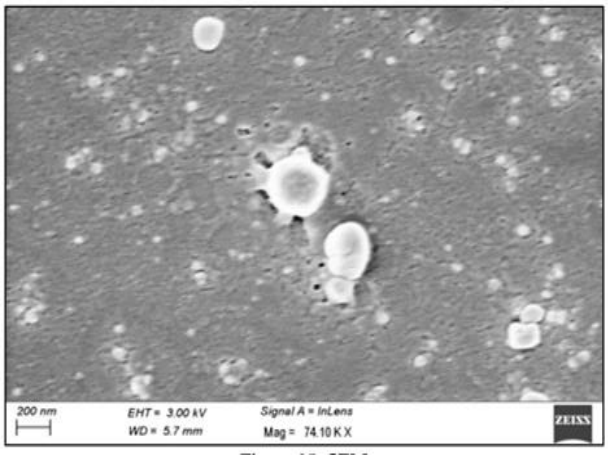

Figure 3: SEM

Results of antimicrobial activity of Niosomes F1 formulation

Table 12: Antimicrobial activity of Niosomes against E.coli and S. aureus

S. No.	Sample Name (mg/ml)	Zone of Inhibition (mm) of *E. coli*	Zone of Inhibition (mm) of *S. aureus*
1.	(Control)	0mm	0mm
2.	A1 Extract (1mg/ml)	5 mm	4 mm
3.	A2 (1.5mg/ml)	10 mm	13 mm

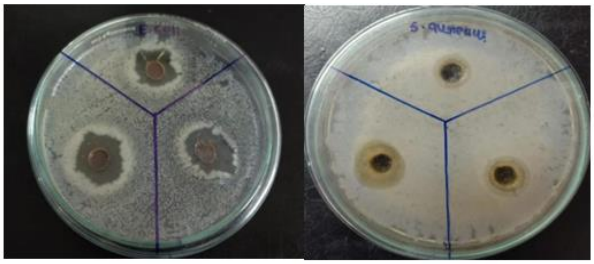

Figure 4: Antimicrobial activity

The antimicrobial activity of the Niosomes F1 formulation was evaluated against E. coli and S. aureus using the well diffusion method. The results indicated that the control showed no inhibition, confirming the absence of inherent antimicrobial activity in the vehicle. The A1 extract at 1 mg/mL exhibited modest zones of inhibition of 5 mm against E. coli and 4 mm against S. aureus, demonstrating preliminary antimicrobial potential. Notably, the A2 formulation at 1.5 mg/mL showed a significant increase in

activity, with zones of inhibition of 10 mm against E. coli and 13 mm against S. aureus, indicating a dose-dependent enhancement of antimicrobial effect. These results suggest that the niosomal encapsulation may improve the delivery and bioavailability of the active components, resulting in enhanced efficacy against both Gram-negative and Gram-positive bacteria. Overall, the data support the potential of the Niosomes F1 formulation as an effective antimicrobial delivery system.

Stability study

The stability study of the F1 niosomal formulation was conducted over 90 days under two different storage conditions: 25°C ± 2°C / 60% ± 5% RH and 40°C ± 2°C / 70% ± 5% RH. Throughout the study period, there were no significant changes observed in physical parameters such as colour, odour, or appearance. The formulation consistently retained its pale green colour, herbal odour, and solid appearance, indicating good physical stability under both storage conditions. In terms of particle size, only minimal variation was noted over 90 days. At 25°C, the size slightly increased from 164.03 nm to 165.25 nm, while at 40°C, it remained nearly constant, from 164.03 nm to 164.14 nm. These minor fluctuations are within acceptable limits and do not indicate any significant aggregation or degradation. Overall, the results confirm that the F1 formulation is physically and colloidally stable for at least 90 days under both ambient and accelerated conditions, making it suitable for further development and storage.

Table 13: Stability Study of Niosomes (F1) formulation

S. No.	Time (days)	250C±2 0C and 60 ± 5% RH				400C±2 0C and 70 ±5% RH			
		Colour	Odour	Appearance	Particle size nm	Colour	Odour	Appearance	Particle size nm
	0	Pale Green	Herbal	Solid	164.03 nm	Pale Green	Herbal	Solid	164. 03 nm
	30	Pale Green	Herbal	Solid	164.09 nm	Pale Green	Herbal	Solid	164.24 nm
	45	Pale Green	Herbal	Solid	164.14 nm	Pale Green	Herbal	Solid	164.17 nm
	60	Pale Green	Herbal	Solid	164.30 nm	Pale Green	Herbal	Solid	164.29 nm
	90	Pale Green	Herbal	Solid	165.25 nm	Pale Green	Herbal	Solid	164.14 nm

SUMMARY AND CONCLUSION

The study focused on the development and characterization of an oral bioavailability-enhancing formulation of Amaranthus Viridis loaded into non-ionic surfactant vesicles (niosomes). Leaves of Amaranthus Viridis were collected and extracted using petroleum ether and methanol, with methanol yielding a significantly higher extract percentage (6.20%) compared to petroleum ether (0.85%). Phytochemical screening of the methanol extract revealed a rich presence of bioactive compounds, including alkaloids, glycosides, carbohydrates, flavonoids, tannins, phenolics, and saponins, confirming the therapeutic potential of the plant. Quantitative analysis showed high levels of phenolic (86.8 mg/g gallic acid equivalents) and flavonoid (42 mg/g rutin equivalents) contents, which are key contributors to antioxidant activity.

The niosomal formulations successfully encapsulated the extract, demonstrated by their pale green color and herbal odor, indicating preservation of extract properties. Particle size analysis identified the NSF 1 formulation as optimal, with a nanosize of 164.03 nm favouring enhanced bioavailability and cellular uptake. Zeta potential measurements supported NSF 1's superior stability (-38.6 mV), and SEM images confirmed spherical and smooth morphology. Antimicrobial assays demonstrated the niosomal formulation's effectiveness against E. coli and S. aureus, indicating potential for therapeutic use. Stability studies of the NSF 1 formulation showed excellent physical and colloidal stability over 90 days under both ambient and accelerated conditions, with minimal changes in particle size, color, odor, and appearance.

In conclusion, Amaranthus Viridis methanolic extract-loaded niosomes, particularly formulation NSF 1, exhibit promising characteristics for improving oral bioavailability and therapeutic efficacy, supported by stable physical properties, effective antimicrobial activity, and high bioactive phytoconstituent content. This formulation holds potential for further development as a natural, effective drug delivery system.

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