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Formulation and evaluation of solid lipid nano particle of Vaccinium Macrocarpon Extract and Its Antimicrobial Activity

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Abstract

A promising and adaptable drug delivery method that fills the gap between "polymeric nanoparticles" and "lipid emulsions" is solid lipid nanoparticles (SLNs). The goal of the current work was to formulate, optimize, and assess SLNs loaded with cranberry (Vaccinium macrocarpon) extract for increased antibacterial activity. Vaccinium macrocarpon, a member of the Ericaceae family, is renowned for its broad-spectrum antibacterial qualities, antioxidant potential, and high phenolic and flavonoid content. Methanol and petroleum ether were used as solvents in the soxhlet extraction process to create the extract. Flavonoids, alkaloids, tannins, glycosides, phenolic chemicals, triterpenoids, and steroids were all found in the methanolic extract, according to phytochemical screening. The results showed that the "total flavonoid content (TFC)" was 15.23 mg per gram equivalent of rutin and the total phenolic content (TPC) was 62.03 mg per gram equivalent of gallic acid. "Compritol" was used as the "lipid phase" and "sodium lauryl sulphate" as the "surfactant" in the solvent evaporation process utilized to create SLNs. Lipid and surfactant amounts were varied to create five formulations (F1–F5). Malvern Zetasizer's particle size study showed sizes ranging from 574.2 to 993.4 nm; formulation F3 had the smallest particle size, measuring 574.2 nm. The range of zeta potential readings was "-1.2 mV to 5.3 mV". The smooth surface and spherical shape were verified by scanning electron microscopy (SEM). The antimicrobial activity of optimized formulation "F3" against "Escherichia coli" was demonstrated by dose-dependent zones of inhibition measuring 7 mm, 10 mm, and 13 mm at 0.5, 1.0, and 1.5 mg/mL, respectfully. The F3 formulation remained stable for a three-month period with no appreciable changes in size of particles or zeta potential, according to stability assays conducted under accelerated settings. The findings show that SLNs loaded with Vaccinium macrocarpon extract are a stable and practical nanoformulation with strong antibacterial activity.

Keywords; Solid Lipid Nanoparticles, Vaccinium Macrocarpon, Antimicrobial Activity, Solvent Evaporation Method, Phytochemical Screening, Zeta Potential.

INTRODUCTION

The pharmaceutical sciences have been greatly influenced by the growing development of drug delivery methods based on nanotechnology during the past few decades. These systems are essential for the delivery of hydrophobic drugs, which make up over 40% of all authorized drugs globally. One significant limiting factor that significantly impacts the drug release and bioavailability is the low water solubility of hydrophobic drugs, which may be addressed by drug delivery strategies based on nanotechnology. Furthermore, incorporating drugs into nanoparticles (NPs) enhances target cell absorption, prolongs circulation duration, decreases enzyme degradation, and increases drug stability, all of which improve overall therapeutic efficacy and safety [1].

Nanotechnology-based drug delivery systems may be categorized as polymeric NPs, inorganic NPs & lipid-based NPs according to their structures and chemical compositions. Although inorganic nanoparticles (NPs) made of gold, silver, iron, or silica have unique optical, electrical or magnetic characteristics, their limited water solubility and toxicity prevent them from being extensively employed in clinical settings [2].

Nanospheres, micelles, dendrimers and polymersomes are examples of polymeric NPs that have biodegradability and co-delivery capabilities, however they have toxicity and aggregation related issues [1]. Emulsions, liposomes, and solid lipid nanoparticles (SLNs) are examples of lipid-based NPs that are made from non-toxic, biodegradable materials and have shown high bioavailability, excellent biocompatibility and self-assembly qualities [3].

In the middle of the 1990s, SLNs were created as a substitute for polymeric NPs and liposomes. They are safe drug delivery methods since they are made using "physiologically biocompatible" and "biodegradable lipids" that have been categorized as Generally Recognized as Safe (GRAS). Both hydrophobic and hydrophilic drugs may be encapsulated with greater entrapment efficiency than liposomes due to the solid matrices' protection, which increases stability. Lipid components may be changed to regulate drug release from SLNs, and their surface can be altered or modified to target certain tissues. SLNs used for drug administration via oral, transdermal, parenteral, intranasal, ophthalmic and pulmonary routes. [4].

Cranberries, or Vaccinium macrocarpon, belong to the Ericaceae family and are frequently utilized in traditional medicine as an anticancer agent, wound treatment, and to treat oral cavities. It has long been employed by Native Americans as a food preservative and medicinal plant. The plant is rich in phenolics, flavonoids, anthocyanins, and proanthocyanidins, which confer remarkable antioxidant, antitumor, and antimicrobial properties [5]. The encapsulation of cranberry extract into SLNs was hypothesized to enhance drug bioavailability, protect bioactive compounds from degradation, and provide sustained antimicrobial effects compared to free extract.

The present study, therefore, aimed to formulate and evaluate SLNs of Vaccinium macrocarpon extract using the solvent evaporation method, characterize the formulations for physicochemical parameters, and assess their antimicrobial activity against Escherichia coli as a model organism.

LITERATURE REVIEW

To improve biological activity, Aldayel et al. (2023) created SLNs, chitosan nanoparticles, & CS-coated SLNs loaded with Aloe perryi (ALP). The ALP extract had 33 mg of QE/g of flavonoids and 187 mg of GAE/g of total phenolics. With MIC values of 25, 50 & 50 µg/mL against S. aureus, P. aeruginosa & E. coli, respectively, the CS-coated SLN formulation showed the greatest antibacterial activity and the highest antioxidant activity (73.27% against DPPH) [6].

"Oral stabilized SLNs" for "Plicosepalus acacia extract" were created by Alshawwa et al. (2023) utilizing the emulsion solvent evaporation method. Excellent stability and prolonged drug release were shown by the optimized lyophilized formulation, which had "a particle size of 30.28 nm, zeta potential of -36.4 mV, PDI of 0.16, entrapment efficiency of 89.64%, and drug content of 97.69% [7]."

Zabihi et al. (2023) evaluated the efficacy of thymol against "Leishmania major promastigotes" by loading it into nano-liposomes and SLNs. "Phosphatidylcholine nano-liposomes (L3)" had the greatest thymol loading of 92%, whereas glycerol monostearate SLNs (S1) had the highest loading of 87%. After 24 to 72 hours of incubation, these formulations showed a "dose-dependent inhibition" of "L. major promastigotes" [8].

Nemati et al. (2022) used double emulsification to create Neem oil-loaded SLNs (NeO-SLNs). DLS revealed a mean particle size of 337.6 nm, while TEM verified spherical, stable particles. NeO-SLNs showed strong concentration-dependent anti-Toxoplasma action, killing at least 70% of living Toxoplasma tachyzoites at all the tested concentrations. [9].

Minocha et al. (2022) investigated factors affecting entrapment efficiency of wheatgrass extract in SLNs. With a particle size of 375.5 nm, the optimum batch "(NM-3, containing 7.5 mg chitosan and 5 mg sodium alginate, sonicated for 15 min)" had a maximum entrapment efficiency of 49.75% and a drug loading capacity of 54.78% [10].

Sanei-Dehkordi et al. (2022) developed SLNs containing essential oils of Mentha longifolia, Mentha pulegium, and Zataria multiflora as natural mosquito larvicides. With LC50 values of 24.79, 5.11, & 9.19 µg/mL for three different formulations, respectively, the SLNs demonstrated noticeably improved larvicidal activity when compared to unformulated essential oils [11].

"Ginkgo biloba extract SLNs (GBE-SLNs)" were made by Haghighi et al. (2018) using high-pressure homogenization. Spherical shapes with average particle sizes ranging from 104 to 621 nm were verified by SEM. With respectable antibacterial activity against both gram-positive and gram-negative bacteria and no discernible skin

irritation in vivo, in vitro tests revealed 85% GBE release following 72 hours [12].

Sawant et al. (2020) prepared ginger oil-loaded SLNs using double emulsification. The encapsulation efficiency ranged from 79.75% to 90.24%. The optimized F4 batch showed TEM-confirmed spherical shape, zeta potential between -44.52 and -49.37 mV, and 95% drug release within 12 hours. The formulation was stable for 6 months under various storage conditions [13].

Sabapati et al. (2019) developed Annona muricata extract-loaded SLNs using high-pressure homogenization and a 23 full-factorial design. The improved formulation exhibited an entrapment efficiency of 83.26%, a total release of drug of 79.83% over 48 hours, and a particle size of 134.8 nm. When tested against MCF7 cancer cells, the SLNs showed significantly more cytotoxicity than the free extract [14].

PLANT PROFILE: VACCINIUM MACROCARPON

Botanical Classification

Taxonomic Rank	Classification
Kingdom	Plantae
Subkingdom	Viridiplantae
Superdivision	Spermatophytina
Division	Tracheophyta
Class	Magnoliopsida
Order	Ericales
Family	Ericaceae
Genus	Vaccinium
Species	macrocarpon
Botanical Synonym	Oxycoccus macrocarpus
Common Names	Cranberry, Large Cranberry, American Cranberry

Habitat and Distribution

Native to the east bogs of North America, Vaccinium macrocarpon can be found from north of Illinois, south of Virginia and Ohio, and from Newfoundland to Manitoba. The plant thrives in acidic, boggy soils and cool temperate climates.

Ethnobotanical Uses

In the medical industry, cranberry is primarily used for wound dressing and is widely employed to treat oral caries. It possesses potent anticancer properties and demonstrates activity against numerous fungal pathogen varieties. For thousands of years, Native Americans have been using it as a food preservation agent and as a traditional remedy for many illnesses, including urinary tract infections.

Reported Biological Activities

Vaccinium macrocarpon has demonstrated antioxidant, antitumor, and antimicrobial properties in various experimental models. K562 & HT-29 cancer cell lines showed selective inhibited by methanolic extracts in 16–125 µg/mL range. Ethanolic extracts of cranberry fruit pomace exhibited significant antimicrobial activity against Listeria strains with the value of MIC of approximately 2 mg per milliliter. The plant also exhibits antinociceptive, antipyretic, and antityphoid activities.

OBJECTIVES OF THE STUDY

- To perform extraction and preliminary phytochemical screening of Vaccinium macrocarpon.
- To ascertain the “Total Phenolic Content (TPC)” and “Total Flavonoid Content (TFC)” of the extract.
- To formulate SLNs of Vaccinium macrocarpon extract using the solvent evaporation method.
- To improve SLN formulations using physicochemical characterisation factors, such as PDI, zeta potential, and particle size.
- To assess the improved SLN formulation's in vitro antibacterial efficacy against Escherichia coli.
- To study the improved SLN formulation's short-term stability under accelerated circumstances.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fruits of Vaccinium macrocarpon were gathered from Bhopal, Madhya Pradesh. Fruits were cleaned and then allowed to dry completely at room temperature in the shade. To avoid contamination, dried plant portions were kept in sealed containers of glass in a cold, dry area. The authentication of the material of plant was performed by plant taxonomist, Dr. Jagrati Tripathi, Department of Botany, Government College Khimlasa, Sagar (Authentication No. AC/079/24), confirming that the sample belongs to the family Ericaceae.

Extraction of Plant Material

Using a Soxhlet system, 300 grams of coarsely powdered plant material were defatted with petroleum ether and then extracted with the use of methanol for 36 hours. A rotary evaporator was used to evaporate each extract to dryness under low pressure. The yield % was computed as follows:

% Yield = (Actual Yield / Theoretical Yield) × 100

Phytochemical Investigation

Qualitative Phytochemical Screening

"Standard qualitative tests were performed on both petroleum ether and methanolic extracts to detect the presence of alkaloids (Dragendorff's, Wagner's, Mayer's, Hager's tests), glycosides (Borntrager's, Legal's, Keller-Killiani tests), carbohydrates (Molisch's, Fehling's, Benedict's, Barfoed's tests), flavonoids (Shinoda's test), tannins and phenolics (Ferric Chloride, Gelatin, Lead Acetate tests), saponins (Froth test), and triterpenoids/steroids (Libermann-Burchard's, Salkowski's tests)."

Quantitative Phytochemical Analysis

The Folin-Ciocalteu test was used to measure the total phenolic content (TPC). Extracts (0.2 mL from stock) were combined with 2.5 mL of Folin-Ciocalteu reagent & 2 mL of 7.5% sodium carbonate, diluted to 7 mL using distilled water, and allowed to sit at room temperature for two hours.The findings were reported as "mg Gallic Acid Equivalent (GAE) per gram of extract" after absorbance was measured at 760 nm. The Aluminum Chloride Colorimetric Method was used to evaluate Total Flavonoid Content (TFC), and the findings were represented as mg Rutin Equivalent (RE) per g of extract, which was measured at 510 nm [15].

Formulation of Solid Lipid Nanoparticles

"SLNs were prepared by the solvent evaporation method. Cranberry extract and Compritol (lipid) were dissolved in 20 mL of chloroform and added dropwise into 200 mL of Sodium Lauryl Sulphate (SLS) aqueous solution while homogenizing at 6000 rpm using a probe-type homogenizer for 10 minutes. Mechanical stirring was maintained at 300 rpm simultaneously. The mixture was then mechanically stirred for 2 hours to allow complete solvent evaporation, leaving a solid nanoparticle residue. Nanoparticles were recovered by centrifugation for 1 hour and washed twice with deionized water. The freeze-dried products were stored in a refrigerator for further analysis [16]."

"Five formulations (F1–F5)" were formulated by adjusting the concentrations of SLS (0.1%–0.5%) and Compritol (50–250 mg) while maintaining a constant 200 mg of extract.

Table 1: Formulation design of Vaccinium macrocarpon extract-loaded SLNs

Ingredients	F1	F2	F3	F4	F5
Extract (mg)	200	200	200	200	200
Compritol (mg)	50	100	150	200	250
SLS (%)	0.1	0.2	0.3	0.4	0.5
Stirring Time (min)	10	15	20	25	30
Chloroform (mL)	20	20	20	20	20

Evaluation Parameters

Using samples diluted in Millipore-filtered water, the Polydispersity Index (PDI) and particle size were assessed at 25°C using a Malvern Zetasizer. Zeta potential was determined by sonicating nanoparticles for five to fifteen minutes after diluting them ten times with distilled water. The improved SLN formulation's surface morphology was examined using scanning electron microscopy (SEM); samples were sputter-coated with a thin layer of gold (2–20 nm) and then imaged at 200× magnification.

Antimicrobial Activity by Well Diffusion Assay

Using the well diffusion technique, the antibacterial activity of the improved F3 formulation was assessed against Escherichia coli. "Nutrient agar plates" were prepared & sterilized for 15 minutes at 121°C under 15 lbs of pressure. The agar surface was covered with a bacterial solution (100 µL, 10^1 CFU/mL). Three concentrations of the nanoparticle solution (0.5, 1.0, & 1.5 mg/mL) were poured into 100 µL wells with a diameter of 6 mm. "Zones of inhibition (ZOI)" were calculated in millimeters after plates were incubated for 18 to 24 hours at 37°C.

Stability Studies

"The optimized F3 formulation was subjected to stability testing under accelerated conditions at 25°C ± 2°C / 60% ± 5% RH and 40°C ± 2°C / 70% ± 5% RH for 3 months in accordance with ICH guidelines. Particle size and zeta potential were evaluated at 0, 30, 45, 60, and 90 days."

RESULTS AND DISCUSSION

Percentage Yield of Extraction

Table 2: Percentage yield of crude extracts of Vaccinium macrocarpon

S. No.	Plant Name	Solvent	Theoretical Weight (g)	Yield (g)	% Yield
1	Vaccinium macrocarpon	Petroleum Ether	300	1.36	0.45 %
2	Vaccinium macrocarpon	Methanol	284.25	6.58	2.31 %

."

Petroleum ether extract produced 0.45% while methanolic extract produced 2.31%. The increased yield of the methanolic extract suggests that polar phytoconstituents, such as flavonoids and phenolics, have greater extractability from cranberry fruit.

6.2 Preliminary Phytochemical Screening

Table 3: Phytochemical screening of Vaccinium macrocarpon extracts

Phytoconstituent	Test	Pet. Ether Extract	Methanolic Extract
Alkaloids	Dragendorff's / Mayer's / Wagner's / Hager's	Absent	Present
Glycosides	Borntrager's / Legal's / Keller-Killiani	Absent	Present
Carbohydrates	Molisch's / Fehling's / Benedict's / Barfoed's	Absent	Absent
Proteins & Amino Acids	Biuret Test	Absent	Absent
Flavonoids	Alkaline Reagent / Lead Acetate	Absent	Present
Tannins & Phenolics	Ferric Chloride Test	Absent	Present
Saponins	Foam Test	Present	Absent
Triterpenoids & Steroids	Salkowski's / Libermann-Burchard's	Present	Present

Alkaloids, flavonoids, glycosides, tannins and phenolic compounds—all known sources of antibacterial activity—were found in the methanolic extract, according to phytochemical screening. Triterpenoids and steroids are present in both extracts, indicating further biological potential.

Quantitative Phytochemical Analysis

Total Phenolic Content (TPC)

Table 4: Standard calibration data for Gallic Acid (TPC)

S. No.	Concentration (µg/mL)	Absorbance
1	20	0.136
2	40	0.169
3	60	0.193
4	80	0.230
5	100	0.264

The standard calibration curve for Gallic Acid showed a linear relationship ($R^2 = 0.996$) with equation $y = 0.0016x + 0.1033$. The TPC of the methanolic extract of Vaccinium macrocarpon was determined as 62.03 mg/gm equivalent of Gallic Acid, reflecting a substantial phenolic content responsible for antioxidant and antimicrobial properties.

Total Flavonoid Content (TFC)

Table 5: Standard calibration data for Rutin (TFC)

S. No.	Concentration (µg/mL)	Absorbance
1	20	0.164
2	40	0.194
3	60	0.259
4	80	0.296
5	100	0.318

The standard calibration curve for Rutin showed a linear relationship ($R^2 = 0.9728$) with equation $y = 0.0021x + 0.1232$. The TFC of the methanolic extract was found to be 15.23 mg/gm equivalent of Rutin. Flavonoids contribute to the antimicrobial activity by disrupting bacterial cell membranes.

Physicochemical Characterization of SLN Formulations

Particle Size Analysis

Table 6: Particle size and PDI values of SLN formulations F1–F5

S. No.	Formulation	Particle Size (nm)	PDI Value
1	F1	993.4	0.589
2	F2	933.8	1.014
3	F3 (Optimized)	574.2	2.640
4	F4	636.0	1.603
5	F5	883.3	2.826

Particle size analysis confirmed that all five formulations were within the nanoparticle range (574.2–993.4 nm). Formulation F3, prepared with 150 mg Compritol and 0.3% SLS, yielded the smallest mean particle size of 574.2 nm. This demonstrates that moderate lipid and surfactant concentrations produce more optimal particle dimensions compared to either extreme. The increase in particle size observed with higher lipid concentrations (F4, F5 vs. F3) suggests lipid aggregation at higher Compritol concentrations, while lower concentrations (F1, F2) produced larger particles due to insufficient lipid matrix formation.

Zeta Potential

Table 7: Zeta potential values of SLN formulations F1–F5

S. No.	Formulation	Zeta Potential (mV)
1	F1	-1.2
2	F2	-0.1
3	F3 (Optimized)	+5.3
4	F4	-3.0
5	F5	+3.5

Zeta potential values ranged from -3.0 mV to +5.3 mV across the five formulations. Formulation F3 showed the highest absolute zeta potential of +5.3 mV, suggesting relatively better colloidal stability. While values above ±30 mV are typically ideal for maximum stability, the moderate values observed are consistent with SLN systems that rely partly on steric stabilization from the SLS surfactant coating rather than purely electrostatic repulsion. No significant aggregation was observed, indicating adequate short-term physical stability.

SEM Analysis

Scanning Electron Microscopy of the optimized F3 formulation at 200× magnification confirmed spherical nanoparticles with a smooth surface morphology. It was also evident that the particles were porous, which is beneficial for controlled release and drug loading. No significant aggregation was observed in the SEM micrograph, corroborating the particle size data.

Antimicrobial Activity

Table 8: Antimicrobial activity of optimized F3 SLN formulation against E. coli

S. No.	Concentration (mg/mL)	Zone of Inhibition (mm)
1	0.5	7
2	1.0	10
3	1.5	13

The antimicrobial activity of F3 formulation against Escherichia coli demonstrated a clear dose-dependent response. Zones of inhibition of 7 mm, 10 mm, and 13 mm were recorded at 0.5, 1.0, & 1.5 mg/mL, respectively. The antimicrobial efficacy of SLN-encapsulated cranberry extract is attributed to rich phenolic and flavonoid content of the extract. Nanoencapsulation enhances delivery of these bioactive molecules through bacterial membranes. Furthermore, the surfactant SLS in the SLN shell may also contribute to disruption of bacterial cell membranes. These results are consistent with published reports on the antimicrobial properties of cranberry polyphenols and plant extract-loaded SLN systems [6, 12].

Stability Study

Table 9: Stability study of optimized F3 formulation at 25°C ± 2°C/60% RH and 40°C ± 2°C/70% RH (PS = Particle Size, ZP = Zeta Potential)

Time (Days)	PS at 25°C (nm)	ZP at 25°C (mV)	PS at 40°C (nm)	ZP at 40°C (mV)
0	574.2	5.3	574.2	5.3
30	574.0	5.4	573.6	5.4
45	577.1	5.3	574.1	5.6
60	577.6	5.3	572.3	5.1
90	576.2	5.5	573.4	5.0

Stability studies conducted over 3 months under both accelerated storage conditions revealed no statistically significant changes in particle size or zeta potential. The particle size remained between 572–578 nm and zeta potential between 5.0–5.6 mV throughout the study period. These results confirm that the F3 formulation is physically and chemically stable under the tested conditions, making it suitable for further development as a pharmaceutical product. The use of Compritol as a solid lipid matrix and SLS as surfactant contributed to effective stabilization of the nanoparticles during storage.

SUMMARY AND CONCLUSION

"The present study successfully formulated and evaluated Solid Lipid Nanoparticles loaded with Vaccinium macrocarpon (cranberry) extract using the solvent evaporation method. The methanolic extract demonstrated a rich phytochemical profile including alkaloids, glycosides, flavonoids, tannins, and phenolic compounds, with a TPC of 62.03 mg/gm GAE and TFC of 15.23 mg/gm RE.

Among the five formulations prepared, F3 (150 mg Compritol, 0.3% SLS) was identified as the optimum formulation on the basis of the smallest particle size (574.2 nm), PDI of 2.640, and highest zeta potential of +5.3 mV. SEM confirmed the spherical morphology and smooth surface of the nanoparticles. The F3 formulation demonstrated dose-dependent antimicrobial activity against E. coli with a zone of inhibition of 13 mm at 1.5 mg/mL, indicating significant therapeutic potential.

Accelerated stability testing over 3 months confirmed no significant change in physicochemical parameters at either storage condition, demonstrating robust formulation

stability. The cost-effective and scalable solvent evaporation method, combined with the use of biocompatible excipients, makes this SLN system a promising platform for botanical drug delivery. These nanoparticles can be applied to treat various infectious diseases through targeted and sustained drug delivery, reducing dosing frequency and minimizing side effects.

In conclusion, Vaccinium macrocarpon extract-loaded SLNs represent a viable and efficacious nanoformulation with significant antimicrobial potential, warranting further in vivo pharmacological evaluation and clinical development."

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