Journal of Molecular Science

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1345-1354
DoI-10.004687/1000-9035.2025.179

Journal of Molecular Science
www.jmolecularsci.com ISSN:1000-9035

Formulation and Evaluation of Spinach-Based Glutathione Mouth Dissolving Granules for Antioxidant and Anti-Aging Potential

Abhimanyu Singh Rathore, Aakash Singh Panwar
SAGE University, Department of Pharmaceutical science, Kailod Kartal, Indore-Dewas By-Pass, Road, Indore, Madhya Pradesh 452020.

Article Information
Received: 23-10-2025
Revised: 14-11-2025
Accepted: 28-11-2025
Published: 23-12-2025

Keywords
Spinach extract, Glutathione, Mouth dissolving granules, Antioxidant, Anti-aging, Hyaluronidase inhibition, Bioavailability

ABSTRACT
The study explores the formulation and evaluation of mouth dissolving granules (MDGs) incorporating glutathione-rich spinach extract for antioxidant and anti-aging effects. Spinach (Spinacia oleracea L.), a known source of polyphenols, flavonoids, and glutathione precursors, was selected for aqueous extraction. Formulations were developed by factorial design, targeting rapid mucosal absorption and improved bioavailability. Glutathione content was quantified using UV-HPLC, and antioxidant potential was assessed through DPPH assay. Anti-aging activity was determined by hyaluronidase inhibition, while microbial and physicochemical properties were evaluated using standard pharmacopeial methods. The optimized formulation exhibited >75% antioxidant activity, 60% hyaluronidase inhibition, and >98% drug release in 1 minute, with strong bioavailability and stability over six months. The outcomes support spinach-based glutathione MDGs as an effective nutraceutical for anti-aging applications, especially in geriatric and pediatric populations. This novel formulation approach offers a promising alternative to synthetic antioxidant therapies.

1. INTRODUCTION:

Herbal medicine, often referred to as botanical medicine or herbalism, involves the therapeutic use of plants and their bioactive constituents. As one of the earliest healing systems practiced by humanity, its origins can be traced to ancient societies such as those of Egypt, China, Greece, and Rome.[1] Additionally, indigenous communities worldwide have developed traditional plant-based treatments based on their native flora. The efficacy of herbal medicine stems from the phytochemicals presents in plants—such as alkaloids, flavonoids, terpenoids, and essential oils—which possess diverse pharmacological actions, including anti-inflammatory, antimicrobial, analgesic, and immunomodulatory effects. [2] Various dosage forms—like infusions, tinctures, powders, topical applications, and encapsulated extracts—are employed depending on the herb and intended clinical outcome. Herbal practitioners often create customized formulations by blending multiple herbs to address individual patient needs.[3]

Spinach (*Spinacia oleracea L.*), a nutrient-dense green leafy vegetable, belongs to the *Amaranthaceou*s family and is botanically related to crops such as beets and Swiss chard. It is widely recognized by various vernacular names across India: *Palak* in Hindi, Gujarati, and Marathi; *Chhurika* in Sanskrit; *Palakh* in Kashmiri; *Palang* in Bengali; *Pasalai* in Tamil; and *Mathrubhumi* in Telugu. Spinach ranks among the top nutrient-rich vegetables consumed globally, especially in the United States, where it competes with broccoli in nutritional value.[48,49]

Spinach can be consumed raw in salads, boiled, frozen, canned, or incorporated into soups, baked items, and traditional dishes. Due to its high nutritional content—including vitamins, minerals, fibre, and antioxidants—spinach plays an essential role in vegetarian diets, particularly in India where plant-based foods constitute the primary source of nutrition.[50]

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1345-1354
DoI-10.004687/1000-9035.2025.179

Fig.1 Spinach leaves

Mouth Dissolving Granules (MDGs) are rapidly disintegrating pharmaceutical or nutraceutical preparations designed to dissolve swiftly in the oral cavity without requiring water. They provide an effective and patient-friendly drug delivery system, particularly suitable for paediatric, geriatric, and dysphagic populations.[52]

Glutathione (GSH) is a tripeptide composed of glutamine, cysteine, and glycine, recognized as one of the most potent endogenous antioxidants. It plays critical roles in detoxification, immune defence, and cellular homeostasis. Endogenously synthesized in the liver, glutathione protects cells from oxidative damage and contributes to overall systemic health.

Functions and Health Benefits:

A. Antioxidant and Detoxification Roles:

Scavenges Free Radicals: Mitigates oxidative damage at the cellular level. Facilitates Liver Detoxification: Involved in conjugation and elimination of heavy metals and xenobiotics.Recycles Antioxidants: Assists in the regeneration of oxidized vitamin C and vitamin E.

B. Dermatological and Anti-Aging Benefits: :Inhibits Melanin Synthesis: Modulates tyrosinase activity, promoting skin lightening.Prevents UV-Induced Damage: Reduces wrinkle formation and enhances dermal elasticity.Promotes Hydration and Collagen Synthesis: Improves skin barrier and texture.

C. Immunomodulatory Activity : Augments:

Immune Cell Function: Enhances lymphocyte proliferation and cytokine response.Reduces Chronic Inflammation: Useful in autoimmune and inflammatory disorders.Strengthens Host Défense: Protects against viral and bacterial pathogens.

D. Neuroprotective and Cognitive Support: ,Mitigates Neurodegeneration: Beneficial in conditions like Parkinson's and Alzheimer's diseases. Enhances Mental Focus and Clarity: Supports mitochondrial function in neurons.

MATERIALS AND METHODS:

Assessment of Quality of Plant Material.

Macroscopic Evaluation: Fresh spinach leaves were examined for organoleptic and morphological characters such as colour, odour, taste, shape, size, surface features, and general appearance as per standard procedures.

Determination of Foreign Matter: Air-dried powdered drug (40 g) was spread in a thin layer and visually inspected. Foreign matter was manually separated, weighed, and the percentage was calculated using the standard formula:Foreign matter (%) = (Weight of foreign matter / Weight of sample) × 100.75–77.

Quantitative Microscopy:

Spinach leaves were subjected to quantitative microscopy and the following indices were determined using reported methods: stomatal index, palisade ratio, vein islet number, and vein termination number.

Proximate Analysis: Proximate analysis of powdered spinach leaves was carried out as per reported procedures.79,80 The following parameters were determined:

Loss on Drying (LOD): A weighed sample (2 g) was dried in an oven to constant weight, cooled in a desiccator, and percentage LOD was calculated as per Indian Pharmacopoeia (2007).81

Total Ash Value: Accurately weighed sample (~2 g) was incinerated in a tared crucible at 500–600°C until carbon-free ash was obtained. The residue was cooled and weighed to calculate total ash as per standard procedure.82

Water-Soluble Ash: Total ash was boiled with water, insoluble residue was collected and ignited, and water-soluble ash was calculated by difference as per pharmacopoeial method.83

Acid-Insoluble Ash: Total ash was boiled with hydrochloric acid, filtered, the insoluble matter was ignited to constant weight, and acid-insoluble ash was calculated.84

Extractive Values: Water-soluble and alcohol-soluble extractive values were determined by maceration, filtration, evaporation, drying at 105°C, and calculation as per Indian Pharmacopoeia (2007).85,86

Foaming Index: Aqueous decoction was prepared, distributed in graded test tubes, shaken for 15 seconds, and foam height was recorded after 15

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1345-1354
DoI-10.004687/1000-9035.2025.179

minutes. Foaming index was calculated using: Foaming index = 1000 / a, where a is the volume (mL) of decoction producing 1 cm foam.87

Extraction of Spinach Leaves: Extraction was carried out at Amsar Pvt. Ltd., Indore. Dried spinach leaves were powdered and macerated (10 g in 100 mL cold distilled water at 4–8°C) for 24 h with shaking. The extract was filtered, centrifuged (5000 rpm, 10 min), and the supernatant was stored at 4°C in amber bottles until analysis.87,88

Phytochemical Screening: Qualitative screening for alkaloids, amino acids, carbohydrates, flavonoids, and glycosides was performed using standard tests (Dragendorff's, Mayer's, Wagner's, Hager's, Millon's, Ninhydrin, Molisch, Barfoed, Seliwanoff, Shinoda, alkaline reagent test, and Fehling-based glycoside tests).90–92

Determination of Total Glutathione Content (HPLC Method): Standard solutions of GSH (5–80 µg/mL) were prepared to construct a calibration curve (Concentration vs Peak area). Spinach extract samples were injected and glutathione content was quantified using the calibration curve. UV detection was used and sample preparation was maintained under cold conditions to prevent oxidation.93–96

HPLC Conditions: C18 column (150 × 4.6 mm, 5 µm); mobile phase: 50 mM phosphate buffer (pH 2.5): methanol (95:5, isocratic); flow 1.0 mL/min; injection 20 µL; detection 210–260 nm; retention time: GSH 4–6 min, GSSG 6–10 min.

Antimicrobial Study of Extract: Antimicrobial activity was evaluated using standard strains (Gram-positive, Gram-negative bacteria and fungi). Agar well diffusion method (CLSI) was performed using different extract concentrations; zone of inhibition was measured. MIC was determined using broth dilution in microtiter plates, followed by MBC/MFC confirmation on agar plates.

Antioxidant Activity (DPPH Assay):DPPH solution (0.1 mM) was prepared in methanol. Spinach extract dilutions (20–100 µg/mL) were mixed with DPPH, incubated for 30 min in dark, and absorbance was measured. % inhibition was calculated using: % Inhibition = [(Acontrol − Asample) / Acontrol] × 100.101–103

Anti-aging Hyaluronidase Inhibitory Assay: Spinach extract (50–400 µg/mL) was incubated with hyaluronidase at 37°C, followed by substrate addition and termination using BSA. Turbidity was measured at 600 nm and inhibition was calculated relative to blank and positive control (catechin/disodium cromoglycate).

Preformulation Study

Organoleptic Properties:Colour, odour, and taste were evaluated to assess general acceptability and detect any abnormal characteristics.

Melting Point; Dried powdered extract was packed in a capillary tube and melting range (onset to clear melt) was recorded using a melting point apparatus.108–110

Calibration Curve: (Ellman's Method): Glutathione standards (0–60 µg/mL) were prepared from stock solution and reacted with DTNB. Absorbance was recorded at 412 nm and calibration curve was plotted.

Solubility Study: Extract (10 mg) was evaluated in various solvents at 25°C and 37°C with vortexing and incubation. Solubility was recorded qualitatively and quantified using UV (≈412 nm after DTNB derivatization, if required).

Partition Coefficient: n-Octanol/water partitioning was performed using separating funnel method, followed by UV estimation (≈412 nm; DTNB derivatization if needed) to calculate distribution of glutathione in both phases.

Drug–Excipient Compatibility:Binary mixtures (1:1) of glutathione extract with each excipient were prepared and stored. Compatibility was assessed using FTIR (4000–400 cm^{-1}) and DSC (25–300°C, 10°C/min, nitrogen purge) by comparing peak shifts/disappearance and thermal changes.

Excipient Profile :Excipient profiles (glutathione extract source and role; citric/ascorbic acid role; mannitol; MCC; SSG; tartaric acid; magnesium stearate; colloidal silicon dioxide) were included briefly to justify selection and functional contribution in formulation.123–130

Formula Optimization (Factorial Design)
Factorial design was applied for optimization of formulation variables and evaluation of optimized mouth dissolving granules.

Table no.1 (Formulation optimization)

S.no.	Glutathione extract (mg)	Mannitol (mg)	MCC (Mg)	Sodium starch Glycolate(mg)	Tartaric acid(Mg)	Mg Stearate (Mg)	L -ascorbic acid (mg)	Colloidal Silicon Dioxide (Mg)
1	1200.00	450.00	200.00	175.00	50.00	22.50	500.00	22.50
2	1100.00	450.00	200.00	150.00	47.50	22.50	500.00	20.00

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1345-1354
DoI-10.004687/1000-9035.2025.179

3	1100.00	450.00	200.00	175.00	50.00	22.50	400.00	22.50
4	1100.00	450.00	175.00	175.00	50.00	25.00	500.00	25.00
5	1000.00	400.00	150.00	150.00	47.50	22.50	450.00	22.50
6	1100.00	450.00	200.00	200.00	47.50	22.50	400.00	20.00
7	1000.00	450.00	175.00	200.00	45.00	22.50	450.00	25.00
8	1200.00	450.00	150.00	175.00	47.50	25.00	450.00	20.00

Method of Preparation:
All ingredients were accurately weighed and sieved (#40). Dry mixing was performed to ensure uniformity. Granules were prepared using dry granulation (preferred for sensitive actives) or wet granulation when required, followed by drying and final blending with magnesium stearate and aerosil. Granules were packed under low humidity conditions.

Evaluation Parameters:
Flow Properties: Angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio were determined using standard pharmacopoeial methods and relevant equations.

Drug Content at Salivary pH: Granules were extracted in simulated salivary fluid (pH 6.8), filtered, and assayed using HPLC (C18 column, UV 210 nm) against a glutathione calibration curve.

In Vitro Bioavailability Setup (Within 1 Minute); Rapid dissolution in simulated salivary fluid was performed with sampling at 30 and 60 seconds, followed by HPLC analysis. Permeation assessment was conducted using Franz diffusion cell with suitable membrane and receptor buffer maintained at 37°C.141–144

Drug Release Study: Dissolution was performed using USP Type II apparatus in phosphate buffer pH 6.8 at 37±0.5°C with sampling at specified intervals, followed by UV/HPLC quantification.

Stability Study: Stability testing was performed as per ICH Q1A(R2) under accelerated and long-term conditions with specified packaging and evaluation parameters at defined time points.

RESULT AND DISCUSSION:

collection and Identification of Plant: Fresh leaves of *Spinacia oleracea* (spinach) were collected directly from a farm and authenticated at Mata Jijabai Govt. P.G. College, Moti Tabela, Indore.

Macroscopic Evaluation: Spinach leaves showed characteristic organoleptic features. Fresh leaves were bright to dark green, while dried leaves appeared dull green to brownish-green. Fresh leaves had a mild characteristic green odor, and dried leaves showed a slightly earthy/grassy smell. The leaves were generally triangular to oblong in shape (2–10 cm depending on age/variety) with a mildly bitter and slightly salty taste (Table 12).

Foreign Organic Matter: Foreign organic matter in spinach leaf powder was found to be 2.548 ± 0.027%, indicating acceptable cleanliness of the crude drug sample (Table 13).

Quantitative Microscopy: Quantitative microscopy supported the identity and quality of the plant material. The stomatal index was 10–15% (Table 14), palisade ratio 4–6 (Table 15), vein islet number 8–12/mm² (Table 16), and vein termination number 4–8/mm² .

Proximate Analysis: Physicochemical parameters indicated good quality and purity of the crude material. Loss on drying was 7.304 ± 0.324% (Table 18), total ash 15.423 ± 0.121% (Table 19), acid-insoluble ash 2.324 ± 0.125% (Table 20), and water-soluble ash 6.341 ± 0.645% (Table 21). The extractive value was 29.341 ± 0.746%, suggesting a good amount of soluble phytoconstituents (Table 22). The foaming index was <100, indicating very low foaming

Heavy Metal Estimation: Heavy metals were within WHO permissible limits. Arsenic (0.22 mg/kg), cadmium (0.03 mg/kg), lead (0.015 mg/kg), mercury (0.002 mg/kg), chromium (0.35 mg/kg), and nickel (0.52 mg/kg) were all found below the specified limits, confirming the safety of plant material for formulation use.

Qualitative phytochemical test: The extracts obtained from successive solvent extraction process were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like Amino acids, carbohydrates, alkaloids, glycosides, phenolics and tannins etc.

Observations and Inference:
- **Alkaloids** showed positive reactions in **Dragendorff's test** (orange-red precipitate) and **Wagner's test** (reddish-brown precipitate), confirming their presence.
- **Flavonoids** were confirmed by **Shinoda test** (pink/red coloration) and **lead acetate test (**yellow precipitate).
- **Amino acids** were detected by **ninhydrin test**

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1345-1354
DoI-10.004687/1000-9035.2025.179

(violet/purple color). **Xanthoproteic test** produced yellow-orange coloration, indicating **aromatic amino acids**, and **Millon's test** gave red color, suggesting **tyrosine** specifically.

- **Tannins and phenolics** were confirmed by **ferric chloride test** (greenish-black coloration), and tannins were further supported by **gelatin test** (white precipitate).
- **Saponins** were present as shown by **foam test** (persistent foam).
- **Glycosides** gave a positive **Keller–Killiani test** (reddish-brown ring at the junction).
- **Proteins** were confirmed by **Biuret test** (violet/purple color).
- **Carbohydrates** were detected by **Molisch's test** (violet ring at the interface).

Determination of Total Glutathione Content, calibration Data

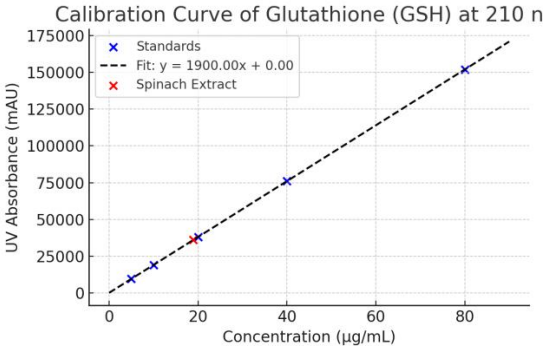

Fig.02 Calibration curve of glutathione

Table no.01 Determination reading

S.no.	Sample	Retention time	Peak area
1	Spinach Extract 1	3.45	36000
2	Spinach Extract 2	3.47	36123
3	Spinach Extract 3	3.46	36154

Table no.02 Summary Output:

S.no.	Parameter	Value
1.	Calibration Equation	y = 1900.00x + 0.00
2.	R^2 Value	0.999
3.	Spinach Extract Peak Area	36092
4.	Calculated Glutathione Content	18.95 µg/mL

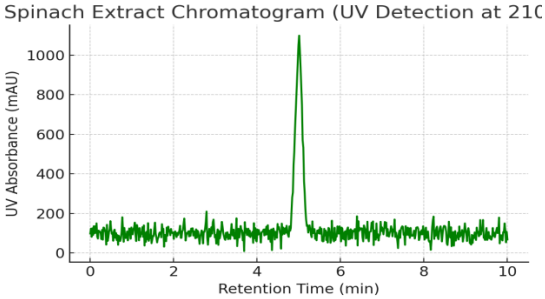

Fig.03 spinach chromatogram

Antimicrobial study of Spinach Extract:

Table no. 03 Zone of Inhibition

S.no.	Microorganism	Positive control	Negative control	Extract 50 mg/mL	Extract 100 mg/mL	Extract 200 mg/mL
1	Staphylococcus aureus	26	0	12	17	22
2	Bacillus subtilis	24	0	10	15	20
3	Escherichia coli	23	0	8	13	18
4	Pseudomonas aeruginosa	22	0	7	12	17
5	Candida albicans	28	0	11	16	21
6	Aspergillus Niger	25	0	9	14	19

Table no.04 Minimum Inhibitory Concentration (MIC)

S.no.	Microorganism	MIC (mg/mL)
1	Staphylococcus aureus	50
2	Bacillus subtilis	50
3	Escherichia coli	100
4	Pseudomonas aeruginosa	100
5	Candida albicans	50
6	Aspergillus niger	100

Table no.05 Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

S.no.	Microorganism	MBC/MFC (mg/mL)
1	Staphylococcus aureus	100
2	Bacillus subtilis	100
3	Escherichia coli	200
4	Pseudomonas aeruginosa	200
5	Candida albicans	100
6	Aspergillus Niger	200

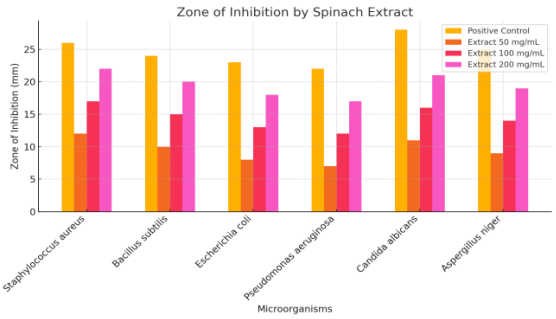

Fig. 05 Zone of inhibition

Determination of antioxidant efficacy of spinach leaf extracts using DPPH:

Table no .06 Determination of antioxidant

S.no.	Concentration (µg/mL)	Absorbance (Extract)	% Inhibition (Extract)	Absorbance (Standard)	% Inhibition (Standard)
1	20	0.642	35.79	0.413	55.92
2	40	0.528	49.43	0.316	65.35
3	60	0.421	60.77	0.226	74.24
4	80	0.332	69.51	0.155	81.48
5	100	0.267	75.32	0.094	87.39

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1345-1354
DoI-10.004687/1000-9035.2025.179

The spinach leaf extract displayed notable antioxidant activity, showing 75.32% inhibition at 100 µg/mL, while the standard ascorbic acid achieved 87.39%. This indicates that spinach leaves are a significant source of natural antioxidants such as glutathione.

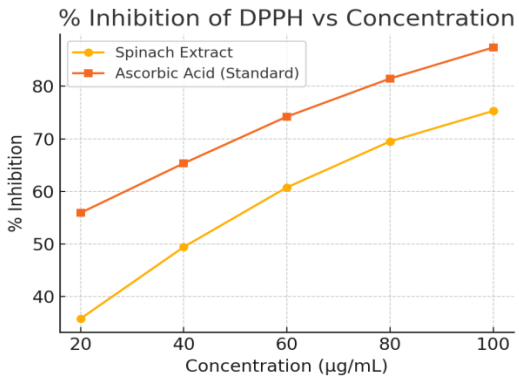

Fig.06.% Inhibition of DPPH vs Concentration

Anti-aging Hyaluronidase Inhibitory Assay for Spinach Extract for Glutathione

Table no.07 Assay setup

S.no.	Concentration (µg/mL)	Absorbance (600 nm)	% Inhibition
1.	Blank (no inhibitor)	0.800	0%
2.	Catechin (positive control)	0.300	62.5%
3.	50 µg/mL extract	0.650	18.75%
4.	100 µg/mL extract	0.520	35.00%
5.	200 µg/mL extract	0.400	50.00%
6.	400 µg/mL extract	0.320	60.00%

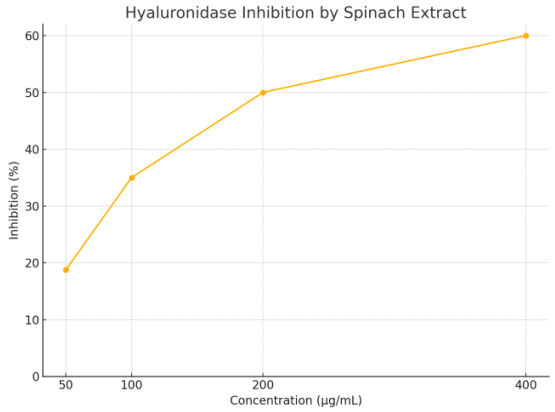

Fig 07. Hyaluronidase inhibition by spinach extract

Preformulating Study:

Table no 08 organoleptic property of extract:

S.no.	Parameter	Value
1.	Colour	Deep green
2.	Odour	slightly grassy smell
3.	Taste	mildly bitter

Table no.09 Melting Point:

S.no.	Sample Name	Onset of melting (°C)	Complete Melting (°C)	Melting Point Range (°C)
1	Dried Spinach Extract Powder	177°C	183°C	177–183°C
2	Dried Spinach Extract Powder	178°C	184°C	178–184°C
3	Dried Spinach Extract Powder	179°C	185°C	179–185°C

The dried spinach extract consistently showed an onset of melting between 177°C and 179°C, and complete melting between 183°C and 185°C.

Table no. 10 The slight variation across samples (±1°C) indicates good reproducibility and purity.

S.no.	Temperature (°C)	Phase observed
1	150	Solid no changes
2	170	Solid no changes
3	178	Onset of melting
4	181	Partial melting observed
5	184	Complete melting (clear liquid)

Table no .11 solubility determination:

S. no.	Solvent	25°C Observation	37°C Observation	Solubility Rating
1	Water	Clear	Clear	+++ (Freely Soluble)
2	Ethanol	Slightly cloudy	Clear	++ (Soluble)
3	Methanol	Clear	Clear	+++ (Freely Soluble)
4	Acetone	Cloudy	Cloudy	+ (Slightly Soluble)
5	Chloroform	Sediment observed	sediment observed	– (Insoluble)
6	DMSO	Clear	Clear	+++ (Freely Soluble)
7	0.1 N HCl	Clear	Clear	++ (Soluble)
8	0.1 N NaOH	Slightly cloudy	Clear	++ (Soluble)

- Water, Methanol, DMSO showed highest glutathione solubility.
- Chloroform showed very low solubility.
- 0.1 N HCl and NaOH showed moderate solubility, indicating glutathione's stability across pH conditions.

Table no .12 Partition Coefficient

S.no	Phase	Absorbance (A)	Using calibration curve	Calculated Concentration (µg/mL)
1	Aqueous	0.522	A = 0.00.0145* C + 0.012	36.00µg/mL
2	octanol	0.192	A = 0.00.0145*	13.24µg/mL

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1345-1354
DoI-10.004687/1000-9035.2025.179

			C + 0.012	

Partition Coefficient (P) = 0.368

Since **P < 1**, glutathione is **more soluble in aqueous phase** than in organic (octanol) phase.

This agrees with glutathione's hydrophilic nature, due to multiple polar groups in its structure

Drug- Excipient compatibility study

Table no.13 FTIR Analysis summary

Sno.	Sample	Peak observation	Interpretation	Compatibility
1	Spinach Extract	Broad O–H, C=O, –SH, aromatic peaks	Herbal matrix baseline	
2	PM1:Spinach+ Mannitol	No change in key peaks	No interaction	Compatible
3	PM2: Spinach + MCC	Peaks retained	No interaction	Compatible
4	PM3: Spinach + SSG	Slight OH broadening (~3400 cm^{-1})	Mild hydrogen bonding	Compatible
5	PM4: Spinach + tartaric Acid	All peaks including –SH retained	No interaction	Compatible
6	PM5: Spinach + Magnesium Stearate	All peaks intact	No interaction	Compatible
7	PM6: Spinach + L-Ascorbic Acid	OH,peak widened	No interaction	Compatible

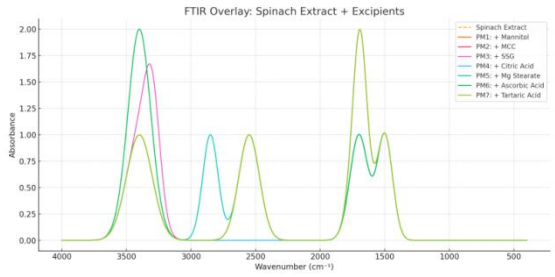

Fig 08. FTIR overlay

DSC Analysis Summary:

Table. No.14 DSC Analysis Summary

S.no.	**Sample**	Thermal Peak	Observation	Compatibility
1	Spinach Extract	~180°C (broad)	Herbal matrix + glutathione	Compatible
2	PM1: Spinach + Mannitol	180°C	No significant shift	Compatible
3	PM2: Spinach + MCC	~178°C	Slight depression, acceptable	Compatible
4	PM3: Spinach + SSG	~175°C (wider)	Broadening observed	Compatible
5	PM5: Spinach + Mg Stearate	~179°C	Similar profile	Compatible
6	PM6: Spinach + Ascorbic Acid	~172°C (wider)	Slight shift and broadening	Compatible
7	PM7: Spinach + Tartaric Acid	180°C	Overlapping stable peak	Compatible

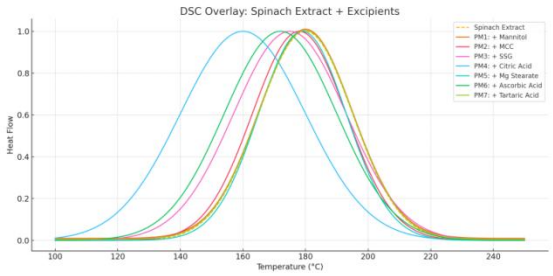

Fig 09 DSC overlay

Flow property:

Table no.15 Flow property:

S.no.	Angle of Repose (°)	Bulk Density (g/mL)	Tapped Density (g/mL)	Carr's Index (%)	Hausner's Ratio	Flowability
1	38.2	0.460	0.540	14.81	1.17	Good
2	36.5	0.475	0.550	13.64	1.16	Good
3	39.8	0.455	0.535	14.95	1.18	Fair
4	41.3	0.440	0.530	16.98	1.20	Fair
5	37.0	0.470	0.540	12.96	1.15	Good
6	42.5	0.430	0.525	18.10	1.22	Passable
7	39.2	0.450	0.530	15.09	1.18	Fair
8	36.9	0.465	0.545	14.68	1.17	Good

Drug content Determination:

The results indicate a strong correlation between glutathione concentration and HPLC peak area across formulation batches. Formulations F1 and F8, each with 1200 mg extract, exhibited the highest glutathione content (190.4 µg and 192.47 µg, respectively) and corresponding AUC values. Notably, batch F7 showed inconsistencies in the total glutathione content, potentially due to error in extract loading or processing. Overall, this analysis confirms effective quantification of glutathione content via HPLC, aiding in the formulation optimization for enhanced bioavailability

Journal of Molecular Science
Volume 35 Issue 4, Year of Publication 2025, Page 1345-1354
DoI-10.004687/1000-9035.2025.179

Table no.16 Drug content Determination

S. no.	Formulation Batch (mg)	Glutathione Extract (mg)	Total Glutathione Content (µg)	Concentration (µg/mL)	HPLC Peak Area (AUC)
1	F1	1200	190.4	1.904	27788.78
2	F2	1100	175.40	1.754	25513.99
3	F3	1100	169.32	1.693	24915.46
4	F4	1100	171.31	1.171	24514.99
5	F5	1000	158.53	1.585	23219.99
6	F6	1100	168.68.	1.686	24867.78
7	F7	1000	1160.56	1.160	23465.86
8	F8	1200	192.47	1.924	27989.89

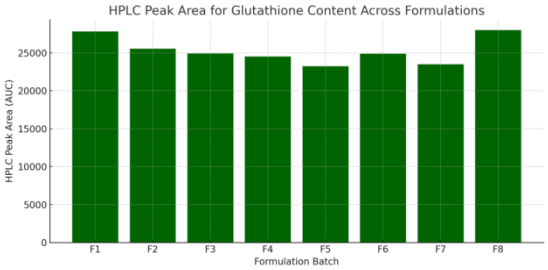

Fig 10 Drug content Bar graph

Bioavailability study:

Table no.17 Bioavailability study:

Formulation Batch	Extract amount mg	% drug release@ 1 min.	% Permeated@ 1 min
F1	1200	97.5	64.2
F2	1100	95.1	61.7
F3	1100	92.8	59.5
F4	1100	94.3	60.9
F5	1000	90.6	56.3
F6	1100	96.2	63.4
F7	1000	89.2	54.1
F8	1200	98.1	65.0

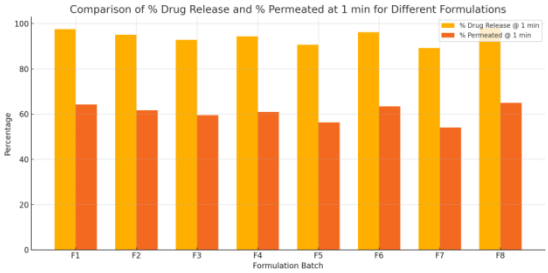

Fig 11 Bioavailability Comparison bar graph

Dissolution study:

Table no.18 Dissolution study:

Formulation Batch	0.25 min %	0.5 min %	1 min %	1.5 min %	2 min. %
F1	41.77	66.66	84.17	90.90	96.80
F2	38.12	64.59	83.54	99.06	93.47
F3	42.88	67.39	82.75	96.13	92.35
F4	38.15	67.04	82.82	92.75	96.36
F5	41.89	60.86	85.14	91.80	99.18
F6	36.62	62.71	88.55	97.83	96.17
F7	43.26	61.13	89.31	92.77	95.10
F8	37.49	64.25	83.92	93.96	98.44

Stability study:

stability study data for all the formulations is now compiled based on ICH Q1A(R2) guidelines (Accelerated: 40°C ± 2°C / 75% RH ± 5%)

one month

Table no.19 Stability study:

Formulation Batch	Appearance	Drug content %	Disintegration Time (sec)	Moisture Content (%)	% Permeated@ 1 min
F1	No change	96.80	34.5	2.23	62.2
F2	No change	93.47	33.7	2.32	64.7
F3	No change	92.35	33.9	2.19	58.5
F4	No change	96.36	32.8	2.13	60.7
F5	No change	99.18	34.6	2.40	56.3
F6	No change	96.17	33.3	2.56	62.4
F7	No change	95.10	34.1	2.24	52.1
F8	No change	98.44	30.8	2.27	66.0

Three months

Table no.20 Stability study:

Formulation Batch	Appearance	Drug content %	Disintegration Time (sec)	Moisture Content (%)	% Permeated@ 1 min
F1	No change	96.80	32.5	2.12	61.2
F2	No change	93.47	33.45	2.28	63.7
F3	No change	92.35	33.66	2.23	59.5
F4	No change	96.36	34.23	2.45	61.7
F5	No change	99.18	33.45	2.34	59.3
F6	No change	96.17	33.12	2.34	61.4
F7	No change	95.10	34.34	2.18	55.1
F8	No change	98.44	30.80	2.27	64.12

Six months

Table no.21 Stability study:

Formulation Batch	Appearance	Drug content %	Disintegration Time (sec)	Moisture Content (%)	% Permeated@ 1 min
F1	No change	96.80	35.5	2.80	63.6
F2	No	93.4	36.7	2.82	65.7

	change	7			
F3	No change	92.35	34.9	2.45	54.5
F4	No change	96.36	36.5	2.67	59.8
F5	No change	99.18	35.3	2.57	58.3
F6	No change	96.17	34.7	2.89	61.4
F7	No change	95.10	35.1	2.24	57.1
F8	No change	98.44	33.17	2.12	69.0

DISCUSSION:

The present work successfully developed and optimized glutathione-enriched herbal mouth-dissolving granules (MDGs) using *Spinacia oleracea* (spinach) as a natural glutathione source. Among eight formulations, F1 and F8 showed the best performance with disintegration within 60 seconds, high glutathione content (HPLC-confirmed), and rapid mucosal permeation. Analytical evaluation (UV and RP-HPLC) validated glutathione richness, while DPPH antioxidant activity and hyaluronidase inhibition supported strong anti-oxidative and anti-aging potential. The formulations also demonstrated meaningful antimicrobial activity, and preformulation studies (FTIR/DSC) confirmed compatibility with excipients. ICH stability studies indicated no significant changes over six months, confirming acceptable shelf stability under suitable packaging conditions. Overall, spinach-based glutathione MDGs represent a fast-acting, user-friendly, and promising prototype for paediatric, geriatric, nutraceutical, and cosmeceutical applications; however, human clinical and pharmacokinetic studies are required to fully validate therapeutic claims and systemic bioavailability.

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