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Formulation and Evaluation of Polyherbal-Based Antiaging Capsules Containing Tinospora Cordifolia, Ocimum Basilicum, and Centella Asiatica

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KEYWORDS

Abstract

This research article explores the formulation and evaluation of polyherbal-based antiaging capsules containing Tinospora cordifolia, Ocimum basilicum, and Centella asiatica. The study encompasses meticulous extraction processes, innovative formulation techniques, and comprehensive evaluations. The leaves of the selected plants underwent a detailed extraction process using advanced techniques, resulting in high-quality plant material. The formulation of capsules involved a systematic approach, employing a Design of Experiment (DOE) methodology. Eight unique batches of capsules were prepared and evaluated, with the optimized formulation (C5) demonstrating excellent in vitro drug release characteristics within 120 minutes.

Chromatographic studies using HPTLC fingerprinting confirmed the presence and stability of active constituents (quercetin, berberine, madecassoside) within the formulated capsules. The RF values and linearity observed at different wavelengths provided additional evidence of the robustness and integrity of the formulated product. The results of the in vitro drug dissolution study revealed a controlled and sustained release pattern for the optimized formulation, demonstrating 76.14% drug release in phosphate buffer pH 6.8 and 62.08% in 0.1 N HCL within 120 minutes.

In conclusion, this research contributes valuable insights to the field of herbal pharmaceuticals, offering a promising avenue for the development of effective antiaging formulations. The combination of innovative formulation strategies and comprehensive evaluations positions these capsules as a noteworthy advancement in the pursuit of herbal-based solutions for antiaging interventions.

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1. Introduction

The relentless pursuit of effective antiaging strategies has become a paramount endeavor in contemporary health and wellness research, fueled by a global fascination with extending both the quality and duration of human life [1]. This study addresses this burgeoning field by focusing on the development and assessment of polyherbal-based antiaging capsules. The formulation integrates extracts from three well-known botanicals: Tinospora Cordifolia, Ocimum Basilicum, and Centella Asiatica [2].

Tinospora Cordifolia, also known as Guduchi, has been traditionally valued in Ayurveda for its immune-boosting and rejuvenating properties. Studies suggest its potential role in promoting skin health by combating oxidative stress and inflammation, key factors in the aging process [3]. Ocimum Basilicum, commonly known as Basil, is celebrated for its antioxidant and anti-inflammatory properties. Rich in essential oils and flavonoids, Basil has shown promise in protecting the skin from environmental stressors and supporting cellular repair mechanisms [4]. Centella Asiatica, or Gotu Kola, has a long history of use in traditional medicine for its woundhealing and anti-inflammatory effects. Modern research emphasizes its potential in enhancing collagen synthesis and skin elasticity, crucial aspects of antiaging interventions [5].

The rationale for combining these botanicals lies in their complementary bioactive compounds, each contributing to a holistic approach in addressing the multifaceted aspects of aging. The synergistic effects of Tinospora Cordifolia, Ocimum Basilicum, and Centella Asiatica hold promise for creating a comprehensive antiaging formulation. This investigation aims not only to contribute to the growing body of knowledge on antiaging strategies but also to rigorously assess the efficacy and safety of this polyherbal approach. Through meticulous formulation and rigorous evaluation, we endeavor to shed light on the potential of these capsules in promoting youthful skin and overall well-being. The outcome of this research has the potential to offer valuable insights into novel antiaging interventions rooted in natural compounds, with implications for both the cosmetic and wellness industries [6].

2. Material and Method

2.1 Collection and Authentication of Plants

The meticulous collection of plant specimens served as the foundational step in this research endeavor. The leaves of Tinospora Cordifolia were thoughtfully gathered from the verdant grounds of Samarth Institute of Pharmacy in Belhe, Pune 412-411. Simultaneously, the leaves of Ocimum Basilicum were sourced from the idyllic village of Otur, Pune 412-411, while the powdered form of dried leaves from Centella Asiatica was procured from the esteemed Shri Brahma Valley Pharmaceuticals in Ambarnath, Maharashtra.

2.2 Standardization of Crude Drug The standardization phase involved a comprehensive validation process encompassing Morphological, Microscopic, Phytochemical, Physical, and HPTLC (High-Performance Thin-Layer Chromatography) fingerprinting evaluations [7].

2.3 Extraction of Plant Material

The extraction of bioactive compounds from plant materials is a crucial step in our research. In this study, we focused on the leaves of Tinospora cordifolia (TC), Ocimum basilicum (OBC), and Centella asiatica (CAT), employing advanced techniques for a meticulous extraction process [8].

Plant Material Preparation:

The leaves of TC, OBC, and CAT were carefully dried under shade for four weeks. Post-drying, the leaves underwent fine powdering using a mortar and pestle, ensuring a homogeneous texture through sieving. For Tinospora cordifolia leaves, 20 grams of the finely powdered plant material underwent methanol extraction using the Soxhlet method. The methanol extract, concentrated through rotary evaporation, yielded a rich concentration of bioactive compounds. Furthermore, the residual marc from the initial extraction underwent an additional extraction with ethanol, enhancing the overall efficiency of bioactive compound extraction [9,10].

2.4. Formulation polyherbal capsule

To formulate granules for capsule preparation, a 3-level 2-factorial design was employed, utilizing Microcrystalline Cellulose (MCC) pH 102, Talc, and Starch as independent variables. The procedure began with the meticulous blending of specified quantities of active ingredients (Quercetin, Berberine, Madecassoside), microcrystalline cellulose, starch, and ethanol. Wet granules were formed by gradually incorporating ethanol into the blend, followed by sieving

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Code	Quarcetin + berberine + Madecassoside	MCC pH 102	Starch	Talc	Ethanol (ml) (q.s)
C1	150	4.0	2.0	1.0	50
C2	150	3.0	3.0	3.0	50
C3	150	4.0	4.0	2.0	50
C4	150	4.0	4.0	2.0	50
C5	150	4.0	3.0	1.0	50
C6	150	5.0	4.0	2.0	50
C7	150	4.0	3.0	3.0	50
C8	150	4.0	2.0	1.0	50

and air-drying to achieve the desired granule size. Talc was added to enhance flow properties [11].

Eight batches of granules, each representing a unique combination of independent variables, were prepared and encapsulated into Size #3 empty hard gelatin capsules. The evaluation process involved rigorous testing, focusing on dissolution and disintegration tests. The optimization phase aimed at identifying the most suitable batch based on these test results. In the Design of Experiment (DOE) formulation phase, a structured approach was employed to formulate granules for hard gelatin capsules. Experimental batches (C1 to C8) were meticulously designed with varying compositions of active ingredients and excipients, allowing for a comprehensive investigation into the influence of key components on granule formulation responses. The detailed composition of each experimental batch is provided in Table 1, outlining quantities of Quercetin, Berberine, Madecassoside, MCC pH 102, Starch, Talc, and Ethanol for each batch. This systematic methodology facilitated a thorough exploration of the interactions between components and their impact on granule formulation [12].

2.5 Evaluation Parameters of Granules The evaluation of granules formed from meticulously designed mixtures involved comprehensive testing to ensure their suitability for capsule formulation. Various parameters, including the angle of repose, particle size, and flow properties, were assessed, with comparisons made against established literature requirements to affirm their appropriateness.

The flow properties of the prepared granules, essential for successful encapsulation, underwent thorough characterization. The following indirect methods were employed [13]: ***Bulk Density Measurements:***

Hausner Ratio: Calculated as the ratio of tapped density to bulk density, providing insights into granule cohesion. Hausner ratio values are indicative of flow properties.

Carr's Index: Obtained by applying the formula (Tapped Density - Fluff Density) / Tapped Density * 100, Carr's Index values below 15 indicate excellent flow, while values between 20 and 30 suggest poor flow.

Angle of Repose:

The angle of repose, determined through the fixed height cone method, serves as a crucial indicator of powder or granule flow properties. It involves pouring the material onto a flat surface, forming a conical heap, and measuring the included angle with the horizontal.

Determination of Granule Particle Size

Uniformity of particle size is a critical factor influencing the therapeutic efficacy of the formulation. Particle size analysis was conducted to interpret granule particle size, ensuring consistency. The granules were passed through different sieves (mesh #40 and #60) to assess their uniformity

Table 1: Experimental batches of granules for hard gelatin capsule.

Batches		Components

Bulk Density, Tap Density, and Carr's Index ***Bulk Density (B.D.):***

In a 50ml measuring cylinder, 15g of polyherbal powdered ingredients were placed, and the initial volume

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(V_0) was recorded. After 50 taps, the powdered volumes (V_{50}) were recorded. Bulk Density (B.D.) is defined as the weight of powder divided by the volume of powder in milliliters.

Tap Density (T.D.):

Calculated as the weight of the powder divided by the volume occupied by the powder after tapping. Tapped density - Fluff density / Tapped density * 100 yields the Carr's Index (C.I.).

Carr's Index (C.I.):

Carr's Index values below 15 indicate excellent flowing material, while values between 20 and 30 suggest poor flowing material.

These meticulous evaluations ensure that the formulated granules align with literature requirements, affirming their suitability for encapsulation into herbal capsules. The comprehensive assessment of flow properties, particle size uniformity, and density characteristics guides the development of a formulation with optimal pharmaceutical attributes. **2.6 Capsule Filling and Packing process** The capsule filling and packing process were executed with meticulous precision, prioritizing environmental conditions to uphold the quality and stability of the final product [14].

Capsule Filling Process:

A manual capsule filling machine was employed for the filling process, ensuring a controlled and standardized procedure. The capsule filling occurred in a regulated environment, maintaining a temperature of 25°C with a relative humidity below 60%. This controlled setting was crucial for preserving the stability of the herbal formulation. Utilizing a manual capsule filling machine allowed for precise and consistent filling, enabling careful monitoring and adjustment to ensure the accuracy of the process. The capsules were filled with preevaluated granules, and a dedusting process was implemented to remove excess powder or particles from the capsule surface, contributing to the overall cleanliness and appearance of the final product [15].

Sealing and Moisture-Free Storage: Following the filling process, the capsules underwent sealing to secure the encapsulated granules within the hard gelatin body. Proper sealing was imperative for maintaining the integrity of the capsules and preventing contamination. Subsequently, the sealed capsules were stored in bottles equipped with silica gel packets. The silica gel served as a desiccant, effectively absorbing moisture from the environment. This moisture-free storage ensured that the herbal capsules remained free from moisture-related degradation, playing a crucial role in preserving the stability and extending the shelf life of the herbal formulation.

2.7 Evaluation of Capsule Formulation The evaluation of herbal capsules is a critical step in ensuring their quality, uniformity, and performance, directly contributing to the verification of dosage consistency, stability, and the capsules' ability to disintegrate as expected [16].

2.7.1 Weight Variation Test:

The weight variation test stands as a pivotal evaluation to assess the uniformity of the powder content in each herbal capsule. In this process, twenty herbal capsules were randomly selected from the batch for evaluation. Each selected capsule underwent precise weighing, and the average weight of the twenty capsules was computed. The percent weight variation was then calculated according to USP (2010) specifications, ensuring that the weight of each herbal capsule fell within 90 and 110 percent of the theoretically estimated weight [17].

2.7.2 Determination of Moisture Content: The moisture content of the capsules is a critical parameter influencing their stability and overall quality. To ascertain this, capsules were carefully stored under specific conditions, maintaining temperatures between 15 and 25 °C and relative humidity levels between 45 and 55%. The maintenance of this moisture level is essential to prevent issues such as flaccidity or brittleness. High humidity can result in flaccid capsules, while low humidity can render them brittle. This meticulous evaluation process ensures the capsules' resilience and quality throughout their shelf life [18].

2.7.3 Disintegration Test:

In the disintegration test, the herbal capsules underwent a critical evaluation of their ability to disintegrate under specific conditions. The tests were conducted at 37 ± 2 °C using a disintegrating apparatus, with distilled water serving as the disintegration medium to simulate physiological conditions. To prevent floating, a disk was strategically placed on each capsule during the test. The evaluation recorded the time taken for all six capsules to disintegrate, leaving only remnants of the gelatin shell on the mesh. These carefully controlled conditions allowed for a comprehensive assessment of the capsules' disintegration characteristics, providing essential data on their quality, uniformity, and performance. This method ensures that the herbal capsules meet the required pharmaceutical standards, confirming their effectiveness and reliability in clinical applications [19].

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2.7.4 In Vitro Dissolution Test

The in vitro dissolution test is a pivotal examination aimed at elucidating the release dynamics of herbal extracts encapsulated within formulated capsules. Conducted using the USP dissolution test apparatus, specifically the Electrolab dissolution tester featuring a basket stirrer, this analysis seeks to simulate and understand the behavior of capsules under physiological conditions [20]. ***Dissolution Test Setup:***

The sophisticated USP dissolution test apparatus, an Electrolab dissolution tester with a basket stirrer, was utilized to ensure controlled and replicable conditions. Two distinct solvents, pH 6.8 Phosphate Buffer and 0.1 N HCl, were employed to mimic different physiological environments. The dissolution test was executed at a regulated temperature of 37.5°C to mirror physiological conditions.

Sampling and Analysis Protocols:

Sampling intervals were strategically chosen at 15, 30, 45, 60, 90, and 120 minutes to capture a comprehensive dissolution profile. At each designated time point, a meticulous 1 mL aliquot of the dissolving media was withdrawn for subsequent analytical procedures. Spectrophotometric analysis, employing the UV-1800 spectrophotometer from Shimadzu, Japan, was adopted for the quantitative assessment of herbal extract release [21]. ***Interpretation of Results:***

Spectrophotometric analysis involved scrutinizing collected samples, diluted to appropriate concentrations, facilitating the quantification of herbal extracts released from the capsules. The UV-1800 spectrophotometer played a pivotal role in measuring absorbance levels, ensuring reliability and precision in result interpretation [22].

2.7.5 Chromatographic Study of Formulated Product

In the chromatographic study of the formulated product, 10 × 10 cm percolated aluminum plates with Silica Gel 60F254 (E. Merck, India) were employed, possessing a thickness of 0.2 mm. To ensure optimal conditions for chromatography, the plates underwent pre-washing with methanol and activation at 60°C for 5 minutes. For the exemplary response, 1 g of extract and an equivalent amount of the final product were accurately weighed and placed into separate iodine flasks. Subsequently, 50 ml of methanol was added to each flask, followed by refluxing for an hour. After filtration, 1-2 cc of the filtrate were concentrated for HPTLC fingerprinting, utilizing varied concentrations of the extract and final product solution (5 and 10 µl). A single band of 6 mm width was applied using the CAMAG LINOMAT V, a modern automatic apparatus [23].

For the development phase, the plate underwent a 60minute development in a CAMAG glass twin-through chamber (10-10 cm) previously soaked with the solvent (Toluene: Ethanol: Formic Acid, 6:3.5:0.5) at 25.2°C and 40% relative humidity. The development distance was set at 8 cm, followed by scanning. Using the CAMAG TLC Scanner-3 and LINOMAT-V, the plate was scanned at UV wavelengths of 255 nm, 267 nm, and 266 nm. The peak area and Rf value for each band of the separated compounds on the plate were recorded for both raw materials and final products. This chromatographic study provides a comprehensive analysis of the formulated product's composition and characteristics [24,25].

3. Results and Discussion

3.1 Collection of Plants

The leaves of Tinospora Cordifolia were sourced from the Samarth Institute of Pharmacy in Belhe, Pune. The leaves of Ocimum Basilicum were collected from the village of Otur, Pune. Additionally, the powdered form of dried leaves from Centella Asiatica was obtained from Shri Brahma Valley Herbaceuticals in Ambarnath, Maharashtra. These collection points were selected for their diverse geographic locations, contributing to a comprehensive selection of plant materials for the study. The detailed origin information ensures transparency and traceability in the research process.

3.2 Standardization of crude Drug

All plant materials underwent a meticulous standardization process, ensuring traceability in the research and guaranteeing the reliability of the study's outcomes. The standardization encompassed comprehensive evaluations, including Morphological, Microscopic, Phytochemical, and physical assessments.

3.2.1 Morphological Evaluation:

Tinospora Cordifolia (TC) Leaves:

The morphological evaluation revealed that TC leaves were simple and alternate, with a broadly ovate to roundish leaf blade measuring 5 to 12 cm in diameter. The lower surface exhibited a slightly pale color, while the upper surface was glaucous. The leaves had an acute tip, sharply acuminate, and a base with a broad sinus. In bulk, the leaves were intensively green, turning yellowish green in mature states. They were characterized by a bitter taste and an indistinct odor.

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Ocimum Basilicum Leaves:

The leaves of Ocimum Basilicum were simple, acute towards the apex, and slightly laviolate in shape. Mature leaves measured 8.4 cm in length, 2.3 cm (middle) x 0.3 cm (base) x 0.1 cm (apex) in width, with a 1.5 cm petiole. Immature leaves were 3.2 cm in length, 1.4 cm (middle) x 0.3 cm (base) x 0.1 cm (apex) in width, with a 1.2 cm petiole. The lower side of the leaf exhibited a prominent midrib and more dotted glandular trichromes compared to the upper surface. The leaves had straight margins, a hairy texture, and spongy characteristics. The upper surface appeared dark green, and the petiole was winged. The leaves emitted a strong aromatic and pleasant fragrance. ***Centella Asiatica Leaves:***

Heart-shaped, small yellow-colored leaves of Centella Asiatica were observed, with dimensions ranging between 2 cm × 3 cm. The leaves were oval in shape, fleshy, orbicular to reniform, and dentate. The petiole was long, smooth on the upper surface, and hairy below. These leaves were borne on pericladial petioles, contributing to the overall distinctive morphology of Centella Asiatica.

3.2.2 Microscopic evaluation

The microscopic evaluation of all plants was done 10x magnification using microscope. The microscopical characteristics of all plants are discussed below. **T. S. of Tinospora Cordifolia Leaf** TS of TC leaf showed anomocytic stomata. Unicellular trichome were present in leaf. Mesophyll was clearly differentiated in palisade layer. Epidermal cells were angular in surface view. Vascular bundles were arranged in a ring shown in figure 1.

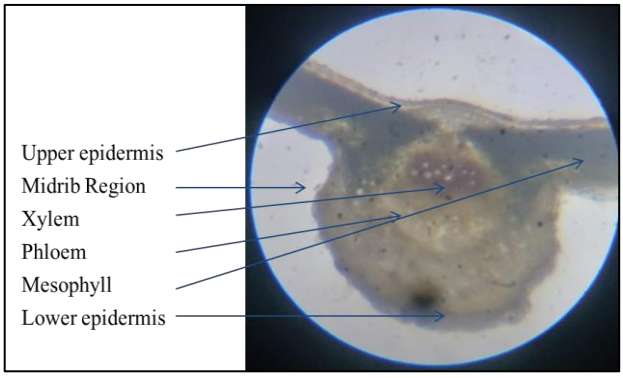

Figure 1: T.S of Tinospora Cordifolia Leaf.

Powder Microscopy of Tinospora Cordifolia Leaf

Powder of leaf of Cordifolia's was passed through sieve no. 60, mounted on glass slide using water, covered with cover slip and viewed under microscope. Powder microscopy showed starch grains that was simple, ovoid or ovoid elliptical. Powder of leaf of T. cordifolia was light to dark brown in color. Pericyclic fibers with a large number of crystal fibers. Bordered pitted vessel, and part of the xylem associated with medullary rays shown in figure 2.

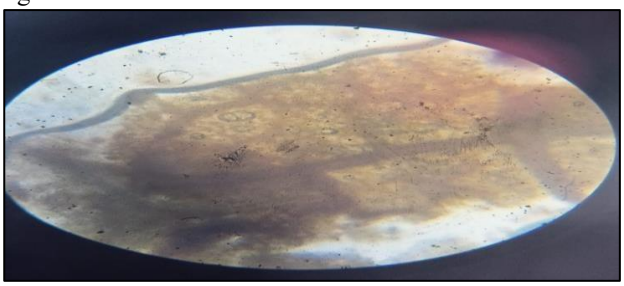

Figure 2: Microscopic image of Tinospora Cordifolia Leaf powder.

T. S. of Ocimum Basilicum Leaf

Mid rib was bowel shaped near about 1.5mm wide. Thin epidermal layer, small epidermal cells. Ground tissue was parenchymatous and cells are small, polygonal and compact. Vascular strand was single, wide and bowl shaped. Xylem and phloem were present. Phloem was seen in small discrete masses showing in figure 3.

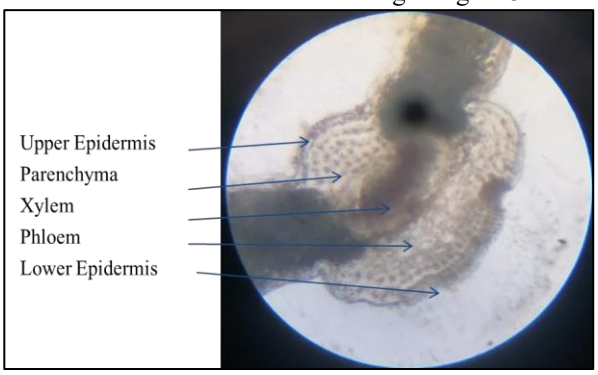

Figure 3: T. S of Ocimum Basilicum Leaf.

Powder Microscopy of Ocimum Basilicum Leaf

Leaves of O. Basilicum, powdered, sieved to obtain fine powder and placed on a clean slide, observed under microscope. A small amount of powder was stained with phloroglucinol solution for few minutes and followed by conc. hydrochloric acid (1:1) in watch glass. Afterwards mounted in glycerin (50%) and observed under microscope. Powder was treated with concentrated H_2SO_4 for the identification of calcium oxalate crystals. The numerous glandular simple trichrome' s of average length 101 µm were observed and shown in figure 4.

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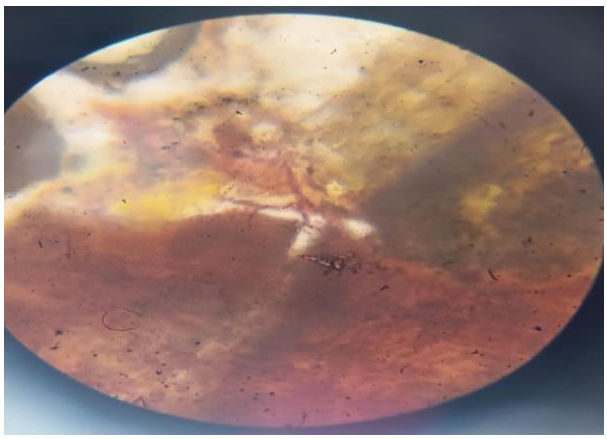

Figure 4: Microscopic image of Ocimum Basilicum Leaf powder.

T. S. of Centella Asiatica

Lamina was found to be differentiated made of epidermis, mesophyll and vascular tissues. Lamina possessed vascular traces, but not differentiated into distinct metaxylem and protoxylem. Both epidermises composed of compactly arranged rectangular cells. Cuticle appeared poorly developed. Noncuticular striated epidermis present. Upper and lower epidermal cells located portion beneath the epidermis sclerenchymatous tissues made of 4-5 layers. Midrib seemed to have a slight depression on the axial side. Midrib composed of collenchyma, mesophyll and vascular bundle shown in figure 5.

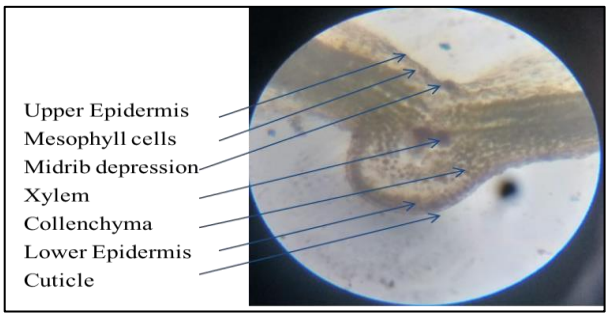

Figure 5: T. S of Centella Asiatica leaf.

Powder Microscopy of Centella Asiatica Leaf

Dried leaves of Centella asiatica analyzed for powder characteristics. Microscopic examination showed fragments of leaf epidermis with venations. Prismatic crystals of calcium oxalate were seen as shown in figure 6.

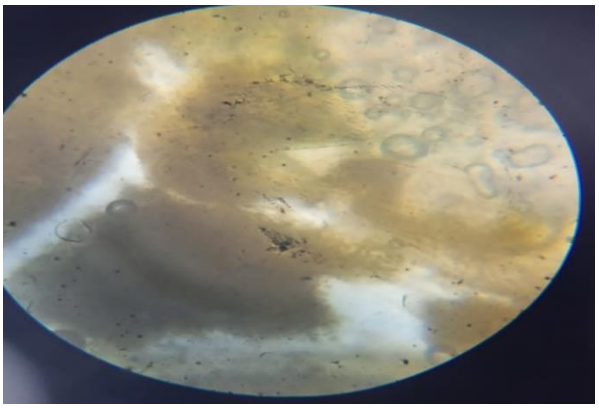

Figure 6: Microscopic image of Centella Asiatica Leaf powder.

3.2.3 Phytochemical Evaluation

A. Qualitative phytochemical analysis of plants The phytochemical screening of all plants showed the presence of moderate secondary metabolite. The occurrence of metabolite indicated in following table. **(+)** =Test positive (presence of constituent) (-) = Test negative (Absent) mentioned in table 2.

Table 2: Summary of Qualitative phytochemical analysis of plants.

Sr. No.	Test	Tinospora Cordifolia		Ocimum Basilicum		Centella Asiatica	
		Pet. Ether	Ethanol	Pet. Ether	Ethanol	Pet. Ether	Ethanol
1	Alkaloids	-	+	-	+	+	+
2	Amino Acids	-	+	-	+	-	-

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9	Tannins	-	+	-	+	-	+
10	Terpenoids	-	+	-	-	-	+
3	Carbohydrates	-	+	-	+	-	-
4	Anthraquinone Glycosides	+	-	-	-	-	-
	Cardiac Glycosides	+	+	+	+	+	+
5	Flavonoids	-	+	+	+	+	+
6	Phenols	-	+	-	+	-	+
7	Saponins	-	-	-	+	-	+
8	Steroids	+	-	-	-	-	+

B. Quantitative phytochemical analysis of plants

A phenolic content and flavonoid content of all plants were determined in quantitative phytochemical analysis. The overall percent content shown in following table 3.

Table 3: Summary of Quantitative phytochemical analysis of plants.

Sr. No	Crude Drug	Foreign Organic Matter (%)	Foreign Organic Matter (%) Rep.	Moisture Content (%)	Moisture Content (%) Rep.	Water soluble Extractive Value (%)	Water soluble Extractive Value (%) Rep.	Alcohol Soluble Extractive Value (%)	Alcohol Soluble Extractive Value (%) Rep.
1.	Tinospora Cordifolia	1.43%	NMT 2%	64%	75 %	9.33%	NMT 11%	2.38%	NMT 3%
2.	Ocimum Basilicum	1.76%	NMT 2%	53%	60%	16.43%	NMT 20%	6.51%	NMT 8 %

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Sr. No	Crude Drug	Total Phenolic Content (%)	Total Phenolic Content (%) Rep.	Total Flavonoid Content (%)	Total Flavonoid Content (%) Rep.
1.	**Tinospora Cordifolia**	4.93%	6.23%	4.56%	8.16%
2.	**Ocimum Basilicum**	6.39%	8.09%	9.86%	8.53%
3.	**Centella Asiatica**	8.11%	8.68%	10.11%	9.17%

3.2.4 Physical Evaluations

A physical evaluation of all plants was done by determining organic matter, moisture content, extractive values, and ash values. All parameters were found to be in limit. The obtained data of physical evaluation study shown in table 4.

Table 4: Summary of physical evaluations of plants.

3.	Centella Asiatica	1.20%	NMT 2%	58%	70%	14.32%	NMT 20%	8.44%	NMT 9%

3.3 Extraction of Plants

The extraction process for all plant materials was meticulously executed using the Soxhlet extraction method, ensuring thorough extraction of bioactive compounds. Subsequently, the extracted materials underwent a comprehensive chromatographic study. For the chromatographic evaluation, Thin Layer Chromatography (TLC) was employed to assess the composition of the extracted compounds from Tinospora Cordifolia, Ocimum Basilicum, and Centella Asiatica. Notably, three distinct spots corresponding to each plant were observed on plate number 1. The first spot indicated Tinospora Cordifolia, the second spot represented Ocimum Basilicum, and the third spot denoted Centella Asiatica. The Relative Front (RF) values for these three plants were found to be 67.1, 65.4, and 69.1, respectively. This chromatographic separation is

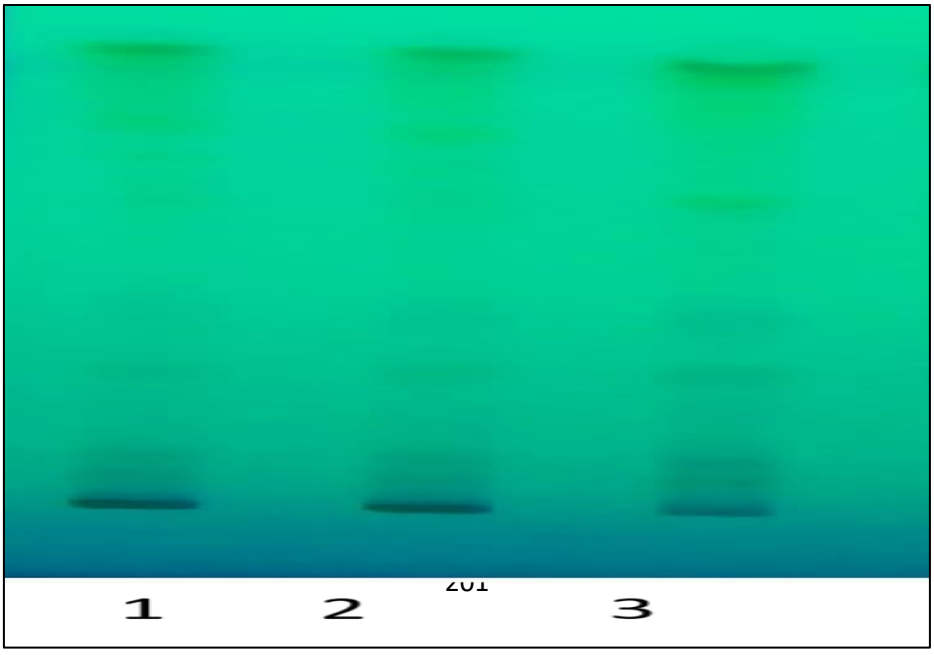

Figure 7: TLC plate of all plants extract.

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illustrated in the accompanying figure 7, offering a visual representation of the distinct compounds present in each plant extract. The precise identification and quantification of these compounds through TLC contribute to a comprehensive understanding of the chemical profiles of Tinospora Cordifolia, Ocimum Basilicum, and Centella Asiatica.

3.4 Evaluation parameters of granules The granules obtained through the formulation process were rigorously evaluated using multiple parameters to ensure their quality and suitability for further processing. The following key parameters were assessed and found to be within the accepted range, as summarized in Table 5. The angle of repose, flow properties, particle size distribution, bulk density, tap density, and Carr's index were all found to be within acceptable ranges. These results indicate the successful formulation of granules with desirable physical characteristics, ensuring their suitability for subsequent processing and encapsulation into capsules. The systematic evaluation of these parameters contributes to the overall quality control of the formulated granules.

Table.5: Summary of evaluation study of granules.

Batches Code	Angle of repose	Flow properties	Particle size (µm)	Bulk density	Tap density	Carr's index
C1	33°	Good	450	0.545	0.617	16
C2	34°	Good	492	0.643	0.736	18
C3	38°	Fair	628	0.486	0.590	20
C4	30°	Excellent	421	0.556	0.598	14
C5	37°	Fair	569	0.589	0.706	19
C6	27°	Excellent	359	0.719	0.785	13
C7	41°	Poor	689	0.606	0.735	21
C8	29°	Excellent	380	0.574	0.656	15

3.5 Capsule Evaluation

3.5.1 Weight Variation Test

The weight variation test, a critical parameter for assessing dosage consistency, was meticulously conducted on all batches of capsules. The test was performed in accordance with the criteria outlined in the United States Pharmacopeia (USP) to ensure compliance with accepted standards. The weight variation test results demonstrate that the average weight per capsule for all batches falls within the specified range, indicating uniformity in the dosage. The standard deviation values, which measure the degree of variability, are also within acceptable limits. These findings affirm the consistency and quality of the formulated capsules, meeting the stringent criteria outlined in the USP for weight variation tests.

3.5.2 Determination of Moisture Content To ascertain the moisture content stability of the capsule formulation, capsules from all batches were carefully stored within a controlled environment, maintaining a temperature range of 15-25°C and a relative humidity range of 45-55%. This controlled storage aimed to mimic real-world conditions and assess the capsules' resistance to environmental factors.

The results of the determination of moisture content indicated no discernible change in the appearance or properties of the capsules across all batches. The capsules exhibited remarkable stability, showing no adverse effects due to variations in temperature and humidity parameters. The consistent "No change" observed in all batches signifies the robust moisture

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resistance of the capsules under the specified temperature and relative humidity conditions. This outcome is crucial for ensuring the long-term stability, quality, and shelf life of the capsule formulation.

3.5.3 Disintegration Test

The disintegration study of the capsules was meticulously conducted in phosphate buffer pH 6.8, employing a disintegration apparatus. The results of the disintegration study, tabulated below in table 6 and as shown in figure 8, adhered to the specifications outlined in the USP/BP, where the acceptable disintegration time ranges from 5 to 30 minutes. Remarkably, none of the samples from the various batches exceeded the specified disintegration time, indicating the capsules' robust disintegration characteristics.

The consistently well-controlled disintegration times across all batches affirm the capsules' ability to rapidly break down in the specified physiological conditions. These findings underscore the adherence to quality standards and highlight the reliability of the formulated capsules in terms of disintegration performance.

Table 6: Disintegration Profile For All Batches.

Batches	Disintegration time (min)	SD
C1	29.05	1.21
C2	26.16	0.35
C3	27.62	1.01
C4	24.22	0.43
C5	23.12	0.34
C6	22.39	0.32
C7	25.08	0.25
C8	24.41	0.32

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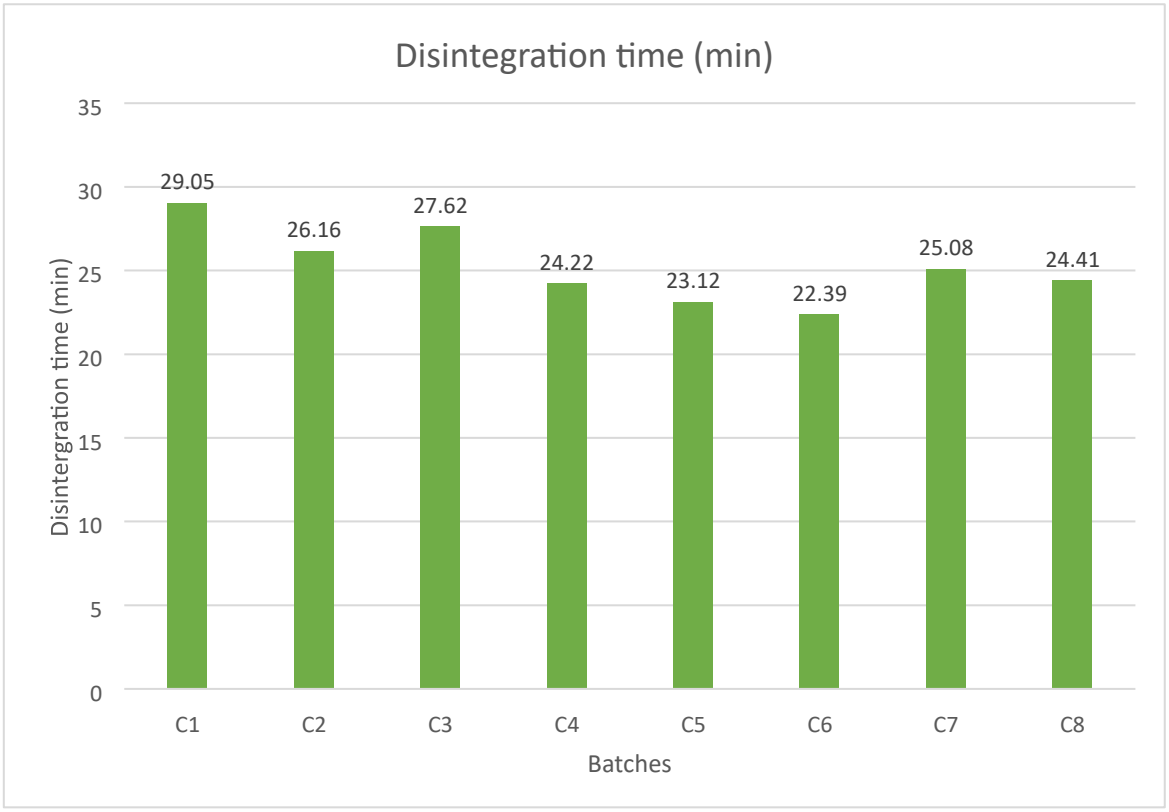

Figure 8: Disintegration Study Of Capsules.

3.6 Experimental Design For Formulation And Optimization Of Capsules

The concentration of factor 1 of MCC pH 102 and factor 2 of Starch and Factor 3 Talc were selected for capsules.

Eight DOE batches of capsules were studied for their corresponding factors and responses 1: Weight variation test and 2: Disintegration test mentioned in table 7.

Table 7: Experimental Design For Formulation.

Batches	Factor-1	Factor-2	Factor-3	Response-1	Response-2
	MCC pH 102	Starch	Talc	Weight variation test	Disintegration test
C1	4.0	2.0	1.0	276.1	29.05
C2	3.0	3.0	3.0	187.5	26.16
C3	4.0	4.0	2.0	215.2	27.62
C4	4.0	4.0	2.0	178.5	24.22
C5	4.0	3.0	1.0	234.6	23.12
C6	5.0	4.0	2.0	254.2	22.39
C7	4.0	3.0	3.0	167.1	25.08
C8	4.0	2.0	1.0	267.7	24.41

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***3.7. In-Vitro Drug* Dissolution Study for Capsule** The in vitro drug release study was conducted on the optimized formulation using phosphate buffer pH 6.4 and 0.1 N HCL as the release media. The optimized formulation, represented by C5, demonstrated a satisfactory drug release profile, with 76.14% release in phosphate buffer and 62.08% in 0.1 N HCL within 120 minutes. This release pattern was deemed favorable, indicating a steady and controlled drug release.

The formulated capsule exhibited a desirable release profile within the specified time frame, adhering to the requirements for pharmaceutical products. The steady drug release pattern observed in the in vitro dissolution study further validates the efficacy and controlled release characteristics of the optimized capsule formulation. The graphical representation of the in vitro drug release profile is provided in table 8 and Figure 9 for enhanced clarity.

Table 8: *In vitro* dissolution study of capsules.

Time (Min)	% CDR Phosphate buffer Ph 6.8	% CDR 0.1 N HCl
0	0	0
5	0.9	0.22
10	8.61	1.24
15	18.77	3.58
30	31.37	7.24
60	47.12	13.665
90	59.37	31.9
120	76.14	62.08

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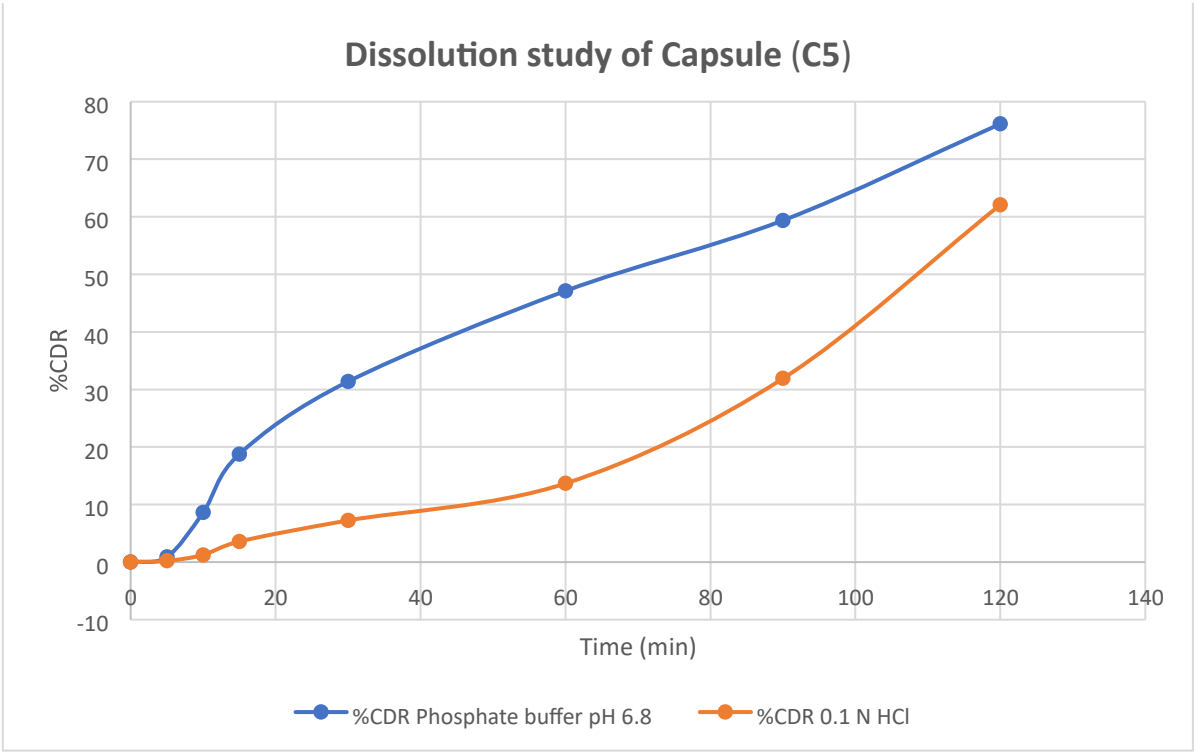

Figure 9*: In-vitro* drug dissolution study of capsules.

3.8 Chromatographic study of formulated products A comprehensive chromatographic evaluation of the formulated optimized capsule was conducted through HPTLC fingerprint study. This study aimed to detect active constituents, namely quercetin, berberine, and madecassoside, at different wavelengths on the HPTLC plate. The results indicated that all constituents remained intact and were not degraded within the capsule shell. The RF (Retention Factor) values for all compounds were determined to be 0.40, 0.60, and 0.69, as illustrated in Figure 10. This suggests a successful encapsulation process, preserving the integrity of the active constituents in the formulated capsules.

Furthermore, the linearity of all compounds was established at wavelengths of 255 nm, 256 nm, and 246 nm, as depicted in Figure 11. This finding reaffirms the stability and consistent presence of the active compounds in the formulated capsules, contributing to the overall quality and reliability of the pharmaceutical product.

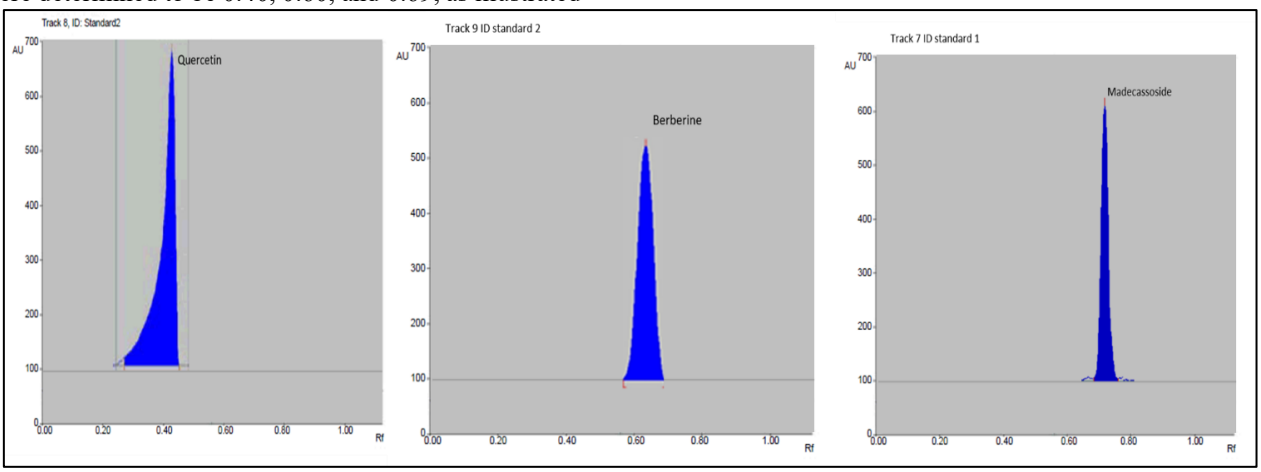

Figure 10: HPTLC plates of active constituent present in formulation.

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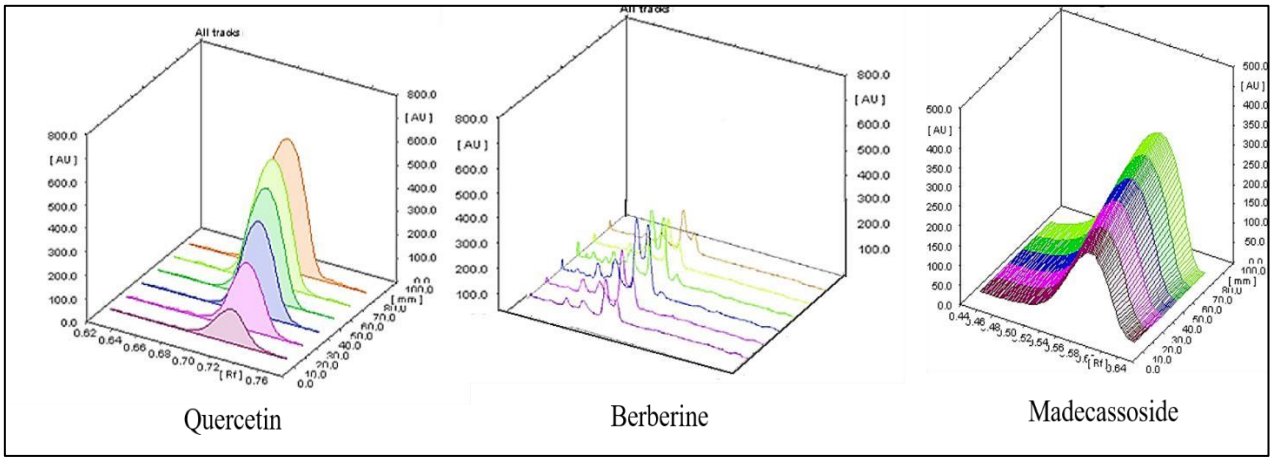

Figure 11: 3D graph of linearity for all compounds.

4. Conclusion

In conclusion, this research article successfully formulated and evaluated polyherbal-based antiaging capsules containing Tinospora cordifolia, Ocimum basilicum, and Centella asiatica. The systematic extraction process, innovative formulation design, and rigorous evaluation techniques culminated in the development of an optimized capsule (C5) with excellent in vitro drug release characteristics. Chromatographic studies confirmed the stability of active constituents, validating the quality and efficacy of the formulated capsules.

The results of the in vitro drug dissolution study revealed a controlled and sustained release pattern for the optimized formulation, demonstrating 76.14% drug release in phosphate buffer pH 6.8 and 62.08% in 0.1 N HCL within 120 minutes. These findings underscore the efficacy of the antiaging capsules in delivering bioactive compounds over a specified timeframe, enhancing their potential therapeutic impact.

This research contributes valuable insights to the field of herbal pharmaceuticals, offering a promising avenue for the development of effective antiaging formulations. The combination of innovative formulation strategies and comprehensive evaluations positions these capsules as a noteworthy advancement in the pursuit of herbal-based solutions for antiaging interventions.

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Conflict of Interest Statement:

The authors declare no conflict of interest in relation to the research, writing, or publication of this article. There are no financial, personal, or professional affiliations that could potentially bias the content or interpretation of the presented findings. This work has been conducted with transparency and objectivity, and the authors are committed to upholding the integrity of the scientific process.

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