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Research Article

Development and Evaluation of a Tri-Herbal Polyherbal Formulation for Immune Health

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ABSTRACT

Immune regulation is closely linked to oxidative and inflammatory balance, and medicinal plants rich in phenolics, flavonoids, and terpenoids offer multifunctional support for this balance rather than acting on a single target. *Withania somnifera* (Ashwagandha), *Ocimum tenuiflorum* (Tulsi), and *Zingiber officinale* (Ginger) each show documented antioxidant, anti-inflammatory, and immunomodulatory potential, but their combined performance in a purpose-built delivery system remains largely unexplored. This study developed and evaluated a tri-herbal polyherbal gummy incorporating hydroethanolic/ethanolic extracts of all three botanicals within a gelatin–pectin matrix. Extracts and the final formulation were screened for phytochemical composition, and the gummies were assessed for weight variation, pH, and stickiness. The formulation retained alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids from the parent herbs, showed low weight variation ($1.57 \pm 0.21\%$), a mildly acidic pH (3.82 ± 0.04), and low adhesiveness (0.018 ± 0.003 mJ), supporting good physical uniformity and reproducibility as a chewable functional dosage form.

INTRODUCTION

In order to preserve host defense and physiological balance, the immune system is a dynamic network that combines cellular, molecular, metabolic, and redox signals. While continuous inflammatory activation can lead to tissue damage and chronic illness, inadequate responses may jeopardize host defense, making appropriate management of immune activation crucial. There is growing

evidence that immune regulation and redox metabolism are intimately related, with alterations in cellular redox status impacting immune-cell activation, differentiation, and function [1]. Thus, a key strategy for promoting good immunological homeostasis is to maintain a suitable balance between oxidative and inflammatory activities. More research is being done on medicinal plants as sources of multifunctional bioactive chemicals that can affect linked biological processes instead

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of just one molecular target. Botanicals that contain phenolics, flavonoids, terpenoids, and steroidal lactones, in particular, have shown anti-inflammatory and antioxidant qualities that may support immunomodulatory effects. Instead of concentrating only on individual phytoconstituents, this multifunctional pharmacological profile offers a justification for investigating botanical combinations. *Withania somnifera* (L.) Dunal, is a medicinal herb of great importance due to its steroidal lactones, particularly withanolides and components related to withaferin. According to recent research, *W. somnifera* has immunomodulatory, anti-inflammatory, and antioxidant properties that may have an impact on both innate and adaptive immune responses. Ashwagandha may influence cytokine responses, T-cell proliferation, macrophage function, and other immune-related activities, according to a 2023 review, encouraging more research on the plant as a possible immunomodulatory herb [2]. Significantly, after *W. somnifera* supplementation in healthy adults, clinical evidence has also shown changes in immunoglobulin, cytokine, T-cell, B-cell, and natural-killer-cell parameters; however, more carefully monitored clinical trials are needed before therapeutic conclusions can be broadly applied [3]. *Ocimum tenuiflorum* L., offers a complementary phytochemical profile. Rosmarinic acid, ursolic acid, oleanolic acid, luteolin, limonene, and other phenolic and terpenoid components are among the plant's many secondary metabolites. *O. tenuiflorum* and its phytoconstituents have significant antioxidant and anti-inflammatory potential, according to a thorough 2024 evaluation that also found openings for more pharmacological and drug-development research [4]. Tulsi is a logical part of a polyherbal mixture meant to target oxidative and inflammatory pathways linked to immune regulation because of these qualities. Through

phenolic components including gingerols, shogaols, paradols, and zingerone, *Zingiber officinale* Roscoe (ginger) offers another pharmacologically significant component. According to a 2024 comprehensive review, ginger has significant anti-inflammatory and antioxidant potential and may have immunomodulatory effects via redox modulation and inflammatory signaling. The mechanisms behind these activities have been suggested to include activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and regulation of nuclear factor-kappa B (NF-κB)-associated pathways [5]. Thus, ginger may enhance the antioxidant and immunomodulatory qualities of tulsi and ashwagandha. Therefore, rather than assuming synergism, the choice of ashwagandha, tulsi, and ginger is based on their phytochemical and pharmacological complementarity. Tulsi provides phenolic and terpenoid antioxidants with anti-inflammatory potential, ashwagandha provides withanolide-rich bioactivity linked to immunological and inflammatory control, and ginger provides antioxidant and inflammatory-pathway modulation connected to gingerol and shogaol [2,4-5]. The biological activity of separate herbs does not, however, guaranty that their combination will be synergistic or additive. Therefore, to ascertain the combination formulation's true antioxidant and immunomodulatory performance, experimental evaluation is crucial. Because functional gummy systems offer a simple and pleasant oral substrate for adding bioactive substances, interest in them has grown in tandem with improvements in botanical pharmacology. Recent reviews highlight the significance of formulation factors such gelling-agent selection, texture, stability, sensory acceptability, moisture, and bioactive retention while describing gummies as an emerging platform for functional compounds, such as plant extracts and antioxidants [6]. Further experimental



research has shown that gummy matrices can effectively contain natural antioxidant extracts, and that formulation adjustment can enhance physicochemical, textural, sensory, and antioxidant properties [7]. These results offer a technological justification for looking into gummies as a multi-herbal preparation delivery method. However, there is still a significant research gap. While ashwagandha, tulsi, and ginger have all shown promise as antioxidants, anti-inflammatory agents, or immunomodulators on their own, little is known about their combined use in a methodically created polyherbal gummy that was especially created and tested for these properties. Furthermore, systematic research is needed to determine how gummy formulation characteristics affect the retention of phytochemical content and biological activity. This gap could be filled by combining modern functional dosage-form development with plant pharmacology. Thus, the goal of this study is to create and assess a polyherbal gummy made of ashwagandha, tulsi, and ginger, with a focus on its physicochemical properties, phytochemical qualities, antioxidant activity, and immunomodulatory potential. The goal of the study is to lay the groundwork for further mechanistic, stability, and clinical research by offering initial scientific proof of the viability of this three-herb combination in a pleasant functional dose form.

2. MATERIALS AND METHODOLOGY

2.1 Plant material

The selected medicinal plants, namely *Withania somnifera* (L.) Dunal (Ashwagandha), *Ocimum tenuiflorum* L. (Tulsi), and *Zingiber officinale* Roscoe (Ginger), were selected based on their reported phytochemical richness and documented antioxidant, anti-inflammatory, and immunomodulatory potential. The roots of *W.*

somnifera, leaves of *O. tenuiflorum*, and rhizomes of *Z. officinale* were used for formulation development. Recent literature emphasizes that the botanical identity, plant part, and phytochemical composition of medicinal plants are important determinants of reproducibility and quality in herbal research [8-10]. The crude plant materials came with pertinent batch/source information and were purchased from a reputable and traceable provider of herbal raw materials. The materials were examined for foreign objects, obvious contamination, discolouration, and other anomalies prior to processing. To reduce the deterioration of thermolabile and photosensitive components, the plant materials were cleaned, rinsed where necessary, and dried under controlled settings shielded from direct sunlight. The dry materials were then individually ground into an appropriate particle size and kept in light-resistant, airtight containers until extraction. Before being added to the polyherbal formulation, each of the three botanicals underwent separate processing to guaranty the traceability of each extract and to make phytochemical and biological analysis easier.

2.2 Preparation of herbal extracts

Before being extracted, the rhizomes of *Zingiber officinale* (ginger), the roots of *Withania somnifera* (ashwagandha), and the leaves of *Ocimum tenuiflorum* (tulsi) were individually cleansed to remove any adhering dirt or extraneous matter, shade-dried, and ground into a powder. A more consistent extraction matrix and easier solvent penetration into the plant tissues are provided by the use of dried and powdered plant material. The polarity and stability of the phytoconstituents meant for further antioxidant and immunomodulatory assessment were considered while choosing the extraction solvent and operating conditions [11-14].



2.2.1 Extraction of *Withania Somnifera*

An ethanol-based solvent system was used to extract the powdered roots of *W. somnifera*. According to recent research, the various phytochemical components of ashwagandha, such as withanolides, phenolic compounds, flavonoids, and associated secondary metabolites, can be effectively recovered using ethanol and hydroalcoholic systems [11]. Root extraction yields of 9.08% with ethanol by reflux extraction, 9.43% with water (9:1, v/v), and 9.51% with water were recorded in the extraction literature compiled by Shinde et al. Microwave-assisted extraction yielded 10.01% with ethanol, 13.75% with water (9:1), and 13.02% with water, while ultrasound-assisted extraction yielded 3.17%, 9.74%, and 11.85% with ethanol, water (9:1), and water, respectively [11]. These results show that the recovery of ashwagandha compounds is significantly influenced by extraction technology and solvent composition. In order to minimize the destruction of thermolabile ingredients, the concentrated extract from the hydroalcoholic extraction of the dried root material for the current polyherbal formulation was dried under low pressure at a regulated temperature. After weighing the dried extract, the yield percentage was computed as follows:

$$\text{Extractive yield (\%)} = [\text{Weight of dried extract (g)} / \text{Weight of powdered plant material (g)}] \times 100$$

Until additional phytochemical and formulation research was conducted, the dried ashwagandha extract was labelled as WSE and kept in a firmly sealed, light-resistant container.

2.2.2 Extraction of *Ocimum tenuiflorum*

Since this solvent solution has been shown in experiments to recover biologically significant phenolic and flavonoid contents from tulsi leaves,

the powdered leaves of *O. tenuiflorum* were extracted using 70% ethanol. An extractive yield of 7.65% w/w based on dried plant material was obtained in a 2022 study using dried *O. tenuiflorum* leaves extracted with 70% ethanol. Rosmarinic acid (147.54 ± 1.79 mg/100 g extract), luteolin (22.70 ± 2.46 mg/100 g extract), and apigenin (31.55 ± 2.82 mg/100 g extract) were identified by LC–ESI–MS/MS characterization of the resultant extract, demonstrating the suitability of hydroethanolic extraction for recovering antioxidant and bioactive components from Tulsi [12]. Thus, 70% ethanol was used to extract the dried powdered leaves, and then the solvent was filtered out under low pressure. The extraction yield was calculated by weighing the semisolid/dry extract that was produced by drying the concentrated extract. The dried Tulsi extract was labelled OTE and kept out of the light and moisture until it was needed. The same gravimetric calculation mentioned above was used to determine the extraction yield. The utility of hydroalcoholic *O. tenuiflorum* extracts in biological investigations is further supported by recent study; a clinically evaluated extract was made from the leaf-rich aerial portions and standardized by HPLC to have $\geq 5\%$ of the Ocimum Bioactive Complex [13]. When the recovery and preservation of several bioactive components are needed, this offers further support for hydroalcoholic extraction.

2.2.3 Extraction of *Zingiber officinale*

An ethanol-based solvent system was used to independently grind and extract the dried rhizomes of *Z. officinale*. Soxhlet extraction for six hours yielded an extractive yield of $17.70 \pm 1.78\%$ with ethanol, compared to $17.93 \pm 6.67\%$ with water, $8.28 \pm 0.48\%$ with ethyl acetate, and $4.82 \pm 0.23\%$ with hexane, according to recent comparative extraction data [14]. Ethanol can be used to



prepare ginger extract for antioxidant and functional studies because of its increased yield and capacity to recover phenolic and other moderately polar compounds. After extraction, the concentrated extract was dried and weighed after the solvent was extracted under low pressure. Until formulation, the dried ginger extract—designated as ZOE—was kept in an airtight, light-proof container. The gravimetric equation was used to determine the % extraction yield:

$$\text{Extractive yield (\%)} = [\text{Weight of dried extract (g)} / \text{Weight of powdered rhizome (g)}] \times 100$$

Before being added to the Ashwagandha–Tulsi–Ginger polyherbal gummy formulation, the acquired WSE, OTE, and ZOE extracts underwent phytochemical characterization and biological evaluation.

2.3 Phytochemical screening

The prepared extracts of *Withania somnifera* (Ashwagandha), *Ocimum tenuiflorum* (Tulsi), and *Zingiber officinale* (Ginger), along with the optimized tri-herbal formulation, were subjected to preliminary qualitative phytochemical screening to identify the major classes of secondary metabolites. The selection of phytochemical classes was based on recent reports describing the presence of phenolic compounds, flavonoids, steroidal lactones, alkaloids and other bioactive constituents in *W. somnifera*, phenolic and flavonoid constituents in *O. tenuiflorum*, and gingerols, shogaols and other phenolic constituents in *Z. officinale* [9,15-16]. For qualitative screening, the extracts were appropriately dissolved in the respective extraction solvent and subjected to individual chemical tests for alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides and carbohydrates. A solvent blank was maintained wherever appropriate. All

observations were recorded based on the development of characteristic colour reactions, precipitate formation or persistent frothing.

2.3.1 Test for alkaloids:

Approximately 1 mL of the extract was acidified with dilute hydrochloric acid and filtered. The filtrate was separately treated with Dragendorff's reagent and Mayer's reagent. Formation of an orange/reddish-brown precipitate with Dragendorff's reagent or a cream-coloured precipitate with Mayer's reagent was considered indicative of alkaloids.

2.3.2 Test for flavonoids:

Approximately 1 mL of extract was treated with a small quantity of magnesium turnings followed by concentrated hydrochloric acid. Development of a pink, red or orange colour was considered a positive reaction for flavonoids.

2.3.3 Test for phenolic compounds:

The extract was treated with a few drops of ferric chloride solution. Development of a characteristic blue, green, violet or dark colour was considered indicative of phenolic constituents.

2.3.4 Test for tannins:

The extract was treated with ferric chloride solution. Formation of a blue-black or greenish-black colour was considered evidence of tannins.

2.3.5 Test for saponins:

Approximately 1 mL of extract was diluted with distilled water and vigorously shaken in a graduated test tube. The formation of persistent froth that remained for several minutes was considered indicative of saponins.

2.3.6 Test for terpenoids:



The extract was mixed with chloroform and concentrated sulfuric acid was carefully added along the wall of the test tube. Formation of a reddish-brown colour at the interface was considered a positive reaction for terpenoids.

2.3.7 Test for steroids:

The extract was subjected to the Liebermann–Burchard reaction by treatment with acetic anhydride followed by concentrated sulfuric acid. Development of a characteristic green or bluish-green colour was considered indicative of steroidal constituents.

2.3.8 Test for glycosides:

The extract was subjected to a suitable glycoside-specific chemical test following the required preliminary treatment. Development of the characteristic colour reaction was considered indicative of glycosidic constituents.

2.3.9 Test for carbohydrates:

The extract was treated with Molisch's reagent followed by careful addition of concentrated sulfuric acid along the wall of the test tube. Formation of a violet-coloured ring at the interface indicated the presence of carbohydrates.

2.3.10 Test for proteins and amino acids:

Proteins and amino acids were screened using the Biuret and ninhydrin reactions, respectively. Development of a violet colour in the Biuret test or a characteristic purple/blue colour with ninhydrin was considered indicative of the respective constituents.

The phytochemical screening was performed in triplicate, and the observations were recorded as absent (–) or present (+) according to the reproducibility of the characteristic reaction. The

same procedure was applied to the individual herbal extracts and the optimized tri-herbal formulation to determine the phytochemical classes retained after formulation. The approach is particularly relevant to the present study because phenolic constituents have been associated with antioxidant capacity in *W. somnifera*, while chemically characterized constituents of *O. tenuiflorum* and *Z. officinale* include compounds with reported antioxidant and inflammation/immunomodulatory relevance.

2.4 Development of polyherbal formulation

The tri-herbal gummy formulation was developed using dry extracts of *Withania somnifera* (Ashwagandha), *Ocimum tenuiflorum* (Tulsi), and *Zingiber officinale* (Ginger) as the active herbal ingredients. A gelatin–pectin hydrocolloid system was selected as the gummy matrix because hydrocolloids are responsible for developing the three-dimensional gel network characteristic of gummy products, while gelatin-based systems can accommodate plant-derived antioxidant extracts [7,17]. Recent studies have demonstrated the feasibility of incorporating bioactive botanical extracts into gelatin-based gummies while retaining antioxidant activity and acceptable rheological properties [17]. Furthermore, recent formulation studies have demonstrated that the concentration of botanical extract and the ratio of gelling agents can significantly influence the physicochemical and textural characteristics of gummy formulations, supporting systematic optimization of these variables [7]. For the preliminary formulation trial, 2.0 g each of Ashwagandha, Tulsi and Ginger dry extracts were used per 100 g of formulation, providing a total herbal extract concentration of 6% w/w. Gelatin (10.0 g) and pectin (2.0 g) were employed as the primary and secondary gelling agents, respectively. Sucrose (25.0 g) and glucose syrup



(20.0 g) were incorporated as sweetening and texture-modifying agents, while citric acid (0.50 g) and sodium citrate (0.30 g) were used as acidulant and buffering components, respectively. Natural flavour and colour were incorporated to improve organoleptic acceptability. The final formulation weight was adjusted to 100 g with purified water. Gelatin was dispersed in purified water and allowed to hydrate for approximately 20–30 min at room temperature. Pectin was separately dispersed in purified water and hydrated under controlled heating with continuous stirring. Sucrose and glucose syrup were combined with purified water and heated to obtain a homogeneous syrup. The hydrated gelatin was subsequently dissolved under gentle heating and mixed with the hydrated pectin dispersion. The sugar syrup was gradually incorporated with continuous stirring to obtain a uniform gummy base. Similar heat-and-pour approaches and gelatin-based matrices have been reported for botanical antioxidant gummy formulations. The gummy mass was allowed to cool to below approximately 45°C before

incorporation of the three herbal extracts. Ashwagandha, Tulsi and Ginger extracts were gradually added under continuous gentle mixing to obtain uniform distribution while minimizing unnecessary thermal exposure of potentially heat-sensitive phytoconstituents. Citric acid, sodium citrate, natural flavour and natural colour were subsequently incorporated. The final mass was adjusted to 100 g with purified water and mixed carefully to minimize air entrapment. The homogeneous mass was immediately transferred into suitable moulds and allowed to set under controlled conditions. The prepared gummies were subsequently evaluated for appearance, weight variation, pH, moisture content, texture, hardness/chewiness, uniformity, total phenolic content, total flavonoid content and antioxidant activity. The use of total phenolic content and antioxidant assays is supported by recent gummy research in which incorporation of botanical extracts produced measurable phenolic content and free-radical-scavenging activity in the finished gummy matrix [17,18].

Table 1. Preliminary trial batches

Batch	Ashwagandha extract (g)	Tulsi extract (g)	Ginger extract (g)	Gelatin (g)	Pectin (g)	Sucrose (g)	Glucose syrup (g)	Citric acid (g)	Sodium citrate (g)	Water
PG1	1.5	1.5	1.5	10	2	25	20	0.50	0.30	q.s.
PG2	2.0	2.0	2.0	10	2	25	20	0.50	0.30	q.s.
PG3	2.5	2.5	2.5	10	2	25	20	0.50	0.30	q.s.
PG4	2.0	2.0	2.0	12	1	25	20	0.50	0.30	q.s.
PG5	2.0	2.0	2.0	10	3	25	20	0.50	0.30	q.s.
PG6	2.0	2.0	2.0	11	2	25	20	0.50	0.30	q.s.
PG7	2.0	2.0	2.0	10	2	25	22	0.50	0.30	q.s.
PG8	2.0	2.0	2.0	10	2	25	20	0.50	0.30	q.s.

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

Table 2. Preliminary phytochemical screening of the developed tri-herbal polyherbal gummy formulation

Phytochemical	Test	Observation	Result
Alkaloids	Mayer's test	Cream precipitate	+
Alkaloids	Wagner's test	Reddish-brown precipitate	+
Flavonoids	Shinoda test	Pink/red colour	+
Flavonoids	Alkaline reagent test	Yellow colour discharged after acidification	+
Phenolics	Ferric chloride test	Bluish-black/green colour	+



Tannins	Gelatin test	Precipitate formation	+
Saponins	Foam test	Persistent froth	+
Terpenoids	Salkowski test	Reddish-brown interface	+
Steroids	Liebermann–Burchard test	Weak greenish colour	±
Glycosides	Keller–Killiani test	Weak characteristic reaction	±
Carbohydrates	Molisch's test	Violet ring	+

Discussion

The developed polyherbal gummy formulation was subjected to preliminary phytochemical screening to determine the major classes of secondary metabolites retained in the finished dosage form. The aqueous/alcoholic extract obtained from the powdered gummy formulation was evaluated using standard qualitative chemical tests. The prepared batch showed positive reactions for alkaloids, flavonoids, phenolic compounds, tannins, saponins and terpenoids, indicating the retention of diverse phytoconstituent classes from the three herbal ingredients. Steroidal and glycosidic constituents were detected weakly/variably, whereas

carbohydrates were strongly detected, which was expected because of the sucrose and glucose syrup incorporated into the gummy base. The presence of phenolic and flavonoid constituents is particularly relevant to the antioxidant potential of the formulation, while terpenoid and alkaloid fractions may contribute to the broader biological activity of the herbal combination. Similar qualitative phytochemical profiling has been applied to polyherbal formulations to establish the presence of major bioactive classes before subsequent quantitative or chromatographic characterization.

3.2 Weight variation

Table 3. Weight variation of the developed tri-herbal polyherbal gummy formulation

Parameter	Result
Number of gummies evaluated	10
Mean weight of gummy	3.18 ± 0.05 g
Minimum weight	3.11 g
Maximum weight	3.26 g
Percentage weight variation	1.57 ± 0.21%
Overall observation	Complies/ acceptable

Discussion

The developed gummies exhibited a mean weight of 3.18 ± 0.05 g, while the individual units showed only a small deviation from the mean weight. The calculated percentage weight variation was 1.57 ± 0.21%, indicating good uniformity among the individual gummy units. The relatively low variability suggests that the gummy mass was distributed consistently during mould filling and setting. The observed uniformity can be attributed

to adequate mixing of the herbal extracts with the gelatin–pectin matrix and controlled deposition of the molten gummy mass into the moulds. Uniform viscosity of the formulation during filling is particularly important because variations in flow or deposition can produce differences in individual gummy weights. Weight variability is generally influenced by formulation characteristics and processing conditions, and control of the manufacturing process is therefore important for achieving consistent unit mass. The low weight



variation also indicates satisfactory reproducibility of the developed formulation at the laboratory scale. Similar evaluation approaches have been reported for gummy dosage forms, where individual gummy weights are compared with the calculated mean to assess batch uniformity. However, weight variation alone should not be interpreted as definitive evidence of active phytochemical dose uniformity. For a polyherbal formulation, confirmation through marker-compound assay or content-uniformity testing would provide stronger evidence that each gummy contains a consistent amount of the herbal actives. USP guidance distinguishes weight variation from content uniformity and specifies when weight variation can be used as an alternative for demonstrating dosage-unit uniformity. Overall, the developed polyherbal gummies demonstrated good unit-to-unit weight consistency, supporting the reproducibility and physical uniformity of the selected formulation.

3.3 pH

The relatively low variability in the measured pH indicates good batch-to-batch uniformity of the prepared formulation. The mildly acidic character of the gummies can primarily be attributed to the incorporation of citric acid, which was used as an acidulant, together with sodium citrate as a buffering component. In addition, the intrinsic acidity of the herbal extracts may have contributed to the final pH of the formulation. The observed pH of 3.82 ± 0.04 falls within the acidic range commonly reported for gummy formulations. Roudbari et al. reported pH values ranging from 3.5 to 6.5 among functional gummy formulations and noted that the pH of approximately 3.5–4.6 was suitable for their gelatin/starch-based gummy system [7].

3.4 Stickiness

The developed gummies exhibited an adhesiveness of 0.018 ± 0.003 mJ, indicating a relatively low adhesive tendency. The low variability among measurements suggests good reproducibility of the surface characteristics of the prepared gummy batch. The observed low stickiness is particularly advantageous for the developed polyherbal gummy because the formulation contains botanical extracts in addition to gelatin, pectin and sugars. The absence of excessive surface tackiness indicates satisfactory matrix formation and suggests that the gummies can be handled and packaged without substantial adhesion-related problems. Overall, the developed formulation demonstrated low adhesiveness and acceptable surface characteristics, supporting its suitability as a chewable gummy dosage form.

4. CONCLUSION

A tri-herbal gummy formulation containing Ashwagandha, Tulsi, and Ginger extracts was successfully developed using a gelatin pectin matrix. Preliminary phytochemical screening confirmed retention of key secondary metabolites alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids from the three herbs in the finished dosage form. The prepared gummies showed good unit-to-unit weight uniformity ($1.57 \pm 0.21\%$ variation), a stable mildly acidic pH (3.82 ± 0.04) suitable for the gelatin-based system, and low stickiness (0.018 ± 0.003 mJ), indicating satisfactory matrix formation and ease of handling. These findings confirm that the gummy dosage form is a technologically feasible and reproducible platform for incorporating multiple botanical extracts, laying a solid foundation for further optimization and quality-control studies of the formulation.



REFERENCES

1. Perpiñán E, Sanchez-Fueyo A, Safinia N. Immunoregulation: the interplay between metabolism and redox homeostasis. *Frontiers in Transplantation*. 2023 Nov 27; 2:1283275.
2. Alanazi HH, Elfaki E. The immunomodulatory role of withania somnifera (L.) dunal in inflammatory diseases. *Frontiers in pharmacology*. 2023 Feb 22; 14:1084757.
3. Tharakan A, Shukla H, Benny IR, Tharakan M, George L, Koshy S. Immunomodulatory effect of Withania somnifera (Ashwagandha) extract—a randomized, double-blind, placebo controlled trial with an open label extension on healthy participants. *Journal of clinical medicine*. 2021 Aug 18;10(16):3644.
4. Bhattarai K, Bhattarai R, Pandey RD, Paudel B, Bhattarai HD. A comprehensive review of the phytochemical constituents and bioactivities of Ocimum tenuiflorum. *The Scientific World Journal*. 2024;2024(1):8895039.
5. Ayustaningwarno F, Anjani G, Ayu AM, Fogliano V. A critical review of Ginger's (Zingiber officinale) antioxidant, anti-inflammatory, and immunomodulatory activities. *Frontiers in nutrition*. 2024 Jun 6; 11:1364836.
6. Tarahi M, Tahmouzi S, Kianiani MR, Ezzati S, Hedayati S, Niakousari M. Current innovations in the development of functional gummy candies. *Foods*. 2023 Dec 25;13(1):76.
7. Roudbari M, Barzegar M, Sahari MA, Gavlighi HA. Formulation of functional gummy candies containing natural antioxidants and stevia. *Heliyon*. 2024 Jun 15;10(11).
8. Gaurav H, Yadav D, Maurya A, Yadav H, Yadav R, Shukla AC, Sharma M, Gupta VK, Palazon J. Biodiversity, biochemical profiling, and pharmaco-commercial applications of Withania somnifera: a review. *Molecules*. 2023 Jan 26;28(3):1208.
9. Bashir A, Nabi M, Tabassum N, Afzal S, Ayoub M. An updated review on phytochemistry and molecular targets of Withania somnifera (L.) Dunal (Ashwagandha). *Frontiers in Pharmacology*. 2023 Mar 29; 14:1049334.
10. Kanai T, Shirahata T, Nakamori S, Koizumi Y, Kodaira E, Sato N, Fuchino H, Kawano N, Kawahara N, Hoshino T, Yoshimatsu K. Development of a determination method for quality control markers utilizing metabolic profiling and its application on processed Zingiber officinale Roscoe rhizome. *Journal of Natural Medicines*. 2024 Sep;78(4):952-69.
11. Shinde S, Balasubramaniam AK, Mulay V, Saste G, Girme A, Hingorani L. Recent advancements in extraction techniques of Ashwagandha (Withania somnifera) with insights on phytochemicals, structural significance, pharmacology, and current trends in food applications. *ACS omega*. 2023 Oct 27;8(44):40982.
12. Srichok J, Yingbun N, Kowawisetsut T, Kornmatitsuk S, Suttisansanee U, Temviriyankul P, Chantong B. Synergistic antibacterial and anti-inflammatory activities of Ocimum tenuiflorum ethanolic extract against major bacterial mastitis pathogens. *Antibiotics*. 2022 Apr 12;11(4):510.
13. CM MG, Murugan SK, Bethapudi B, Purusothaman D, Mundkinajeddu D, D'Souza P. Ocimum tenuiflorum extract (HOLIXERTM): Possible effects on hypothalamic–pituitary–adrenal (HPA) axis in modulating stress. *PloS one*. 2023 May 4;18(5):e0285012.
14. Spyrou A, Batista MG, Corazza ML, Papadaki M, Antonopoulou M. Extraction of high value products from Zingiber officinale roscoe (Ginger) and utilization of residual biomass. *Molecules*. 2024 Feb 16;29(4):871.
15. Polumackanycz M, Petropoulos SA, Śledziński T, Goyke E, Konopacka A, Plenis A, Viapiana A. Withania somnifera L.: phenolic compounds composition and biological activity of commercial samples and its aqueous and hydromethanolic extracts. *Antioxidants*. 2023 Feb 22;12(3):550.



16. Russo R, Costa MA, Lampiasi N, Chiaramonte M, Provenzano A, Mangione MR, Passantino R, Zito F. A new ginger extract characterization: Immunomodulatory, antioxidant effects and differential gene expression. *Food Bioscience*. 2023 Jun 1; 53:102746.
17. Aiello F, Caputo P, Oliviero Rossi C, Restuccia D, Spizzirri UG. Formulation of antioxidant gummies based on gelatin enriched with citrus fruit peels extract. *Foods*. 2024 Jan 19;13(2):320.
18. Renaldi G, Junsara K, Jannu T, Sirinupong N, Samakradhamrongthai RS. Physicochemical, textural, and sensory qualities of pectin/gelatin gummy jelly incorporated with *Garcinia atroviridis* and its consumer acceptability. *International Journal of Gastronomy and Food Science*. 2022 Jun 1;28:100505.

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