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A COMPREHENSIVE REVIEW ON DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR ANTIDIABETIC PHYTOCONSTITUENT EVALUATION IN WITHANIA COAGULANS-LOADED HERBAL GUMMIES

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ABSTRACT
Withania coagulans is a medicinal plant traditionally used in South Asia, and its bioactive phytoconstituents, particularly withanolides and flavonoids, have demonstrated considerable antidiabetic potential. However, the translation of these benefits into standardized, patient-friendly oral dosage forms such as herbal gummies remains poorly investigated. The present systematic review was designed to critically synthesize and evaluate the existing evidence on the development and analytical validation of *W. coagulans*-loaded gummy formulations for antidiabetic applications. Our methodology followed the PRISMA guidelines, and we conducted a comprehensive literature search across multiple scientific databases. We included original peer-reviewed studies that addressed the phytochemical characterization of *W. coagulans*, its antidiabetic evaluation, or the formulation of herbal gummies, and we excluded studies on other *Withania* species or non-oral dosage forms. Two independent reviewers screened the records, extracted data on extraction methods, analytical validation parameters, formulation ingredients, and antidiabetic outcomes, and assessed the methodological quality of the included studies. The results revealed a pronounced disconnect in the literature. While substantial evidence supports the antidiabetic activity of *W. coagulans* extracts through enzyme inhibition and antioxidant assays, we found no validated analytical methods for quantifying key phytoconstituents within a gummy matrix. Furthermore, existing formulation studies focused on other herbs, and no standardized protocol for incorporating *W. coagulans* while maintaining phytoconstituent stability was identified. The review therefore highlights critical gaps, including the absence of stability-indicating chromatographic methods for complex gummy matrices and the lack of any in vivo or clinical translation studies. We conclude that future research must prioritize the development of validated analytical protocols, systematic formulation optimization, and controlled preclinical trials to bridge the gap between raw plant characterization and a ready-to-market, evidence-based antidiabetic nutraceutical.

KEYWORDS: Withania coagulans, Herbal Gummies, Antidiabetic Phytoconstituents, Analytical Method Validation, Meta-Analysis.

INTRODUCTION

Diabetes mellitus represents a global health crisis, affecting an estimated 537 million adults worldwide according to the International Diabetes Federation, with projections suggesting a continued rise in prevalence (Atlas, 2021). The disease, characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both, leads to severe long-term complications including neuropathy, nephropathy, retinopathy, and cardiovascular disease (Association, 2010). Conventional pharmacological interventions, such as metformin, sulfonylureas, and insulin, are effective but often associated with adverse effects, high costs, and limited accessibility in resource-constrained settings (Mori & Sekihara, 2002). Consequently, there is a growing interest in complementary and alternative therapies, particularly those derived from medicinal plants, which offer a rich source of bioactive molecules with potential antidiabetic properties (Mukesh & Namita, 2013).

Among the numerous medicinal plants investigated for antidiabetic activity, *Withania coagulans* (syn. *Puneeria coagulans*), commonly known as “Indian cheese maker” or “paneer dodi,” stands out due to its long history of use in traditional Ayurvedic and Unani medicine (Sinoriya et al., 2024). This small shrub, native to the arid regions of South Asia and the Middle East, has been traditionally employed for the management of diabetes, gastrointestinal disorders, and inflammatory conditions (M. Khan et al., 2021). The fruits of *W. coagulans* contain a unique array of phytoconstituents, primarily withanolides such as coagulansin A, coagulansin B, and withaferin A, along with flavonoids, phenolic acids, and steroidal lactones (Mathur et al., 2011). These compounds have been shown to exhibit multiple antidiabetic mechanisms including inhibition of α-amylase and α-glucosidase enzymes, enhancement of glucose uptake in adipocytes and muscle cells, stimulation of insulin secretion from pancreatic β-cells, and potent antioxidant activity that mitigates oxidative stress associated with diabetes (Maher et al., 2020). Despite this promising phytochemical and pharmacological profile, the clinical translation of *W. coagulans* into a standardized, patient-friendly dosage form remains an unmet challenge.

The development of oral dosage forms for herbal medicines is inherently complex. Herbal gummies have recently emerged as a popular alternative to conventional tablets and capsules, particularly for patients who experience swallowing difficulties or prefer a palatable, chewable delivery system (Yadav et al., 2024). Gummy formulations offer several advantages: they can mask the bitter taste of many phytochemicals, provide a controlled release profile if appropriately designed, and are generally perceived as a food product, thereby improving patient compliance (Yadav et al., 2024). However, the incorporation of a complex plant extract like *W. coagulans* into a gummy matrix presents significant technical hurdles. The gummy matrix, typically composed of gelatin or pectin, sugars, humectants, and acidulants, may interact with the phytoconstituents, affecting their chemical stability, release kinetics, and ultimately their bioavailability (Shahidi & Pan, 2022). For instance, the acidic pH of gummy formulations may catalyze the hydrolysis of withanolides, while heating during preparation can degrade thermolabile flavonoids (Patil et al., 2010).

Moreover, the presence of sugars may pose a paradox for antidiabetic formulations, requiring careful optimization of sweetener selection and concentration. Hence, the formulation of an efficacious *W. coagulans* gummy requires a systematic approach that balances phytoconstituent stability, sensory acceptability, and therapeutic efficacy.

A critical research gap that emerges from this context is the absence of validated analytical methods for quantifying the key antidiabetic phytoconstituents of *W. coagulans* within a gummy matrix. While numerous studies have characterized the phytochemical profile of *W. coagulans* extracts using high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS) (Ali et al., 2015), these methods were developed for raw plant materials or simple solvent extracts, not for complex processed formulations. The gummy matrix introduces additional interactions and potential interferences that necessitate a dedicated method development and validation process.

According to international guidelines such as those from the International Council for Harmonisation (ICH) and the Association of Official Analytical Collaboration (AOAC), analytical methods for herbal drug products must demonstrate specificity, accuracy, precision, linearity, range, sensitivity (detection and quantitation limits), and robustness (Food & Administration, 2005). Furthermore, stability-indicating methods are required to assess the degradation of phytoconstituents under formulation and storage conditions. To date, no peer-reviewed publication has reported the development and full validation of such a method for *W. coagulans* in a gummy dosage form. This gap represents a substantial barrier to quality control, batch-to-batch consistency, and regulatory approval of *W. coagulans* gummies as a nutraceutical or phytopharmaceutical product.

Another significant void in the literature is the lack of systematic formulation studies that integrate phytochemical characterization, antidiabetic efficacy, and dosage form design for *W. coagulans*. Existing research remains fragmented: phytochemical studies focus on raw extracts, pharmacological studies use crude extracts or isolated compounds often administered in suspension or solution, and formulation studies on herbal gummies predominantly involve other herbs such as *Ocimum sanctum* (tulsi), *Curcuma longa* (turmeric), or *Withania somnifera* (ashwagandha) (Dengane et al., 2026). For *Withania coagulans* specifically, we have not identified any published work that describes the complete pipeline from extract preparation, through analytical method development, to gummy formulation and validation. This disconnect impedes the translation of preclinical evidence into a viable product and leaves a critical evidence void regarding the stability and bioactivity of *W. coagulans* phytoconstituents in a gummy matrix. Moreover, no in vivo studies or clinical trials have been conducted to evaluate the antidiabetic efficacy of *W. coagulans* gummies, further underscoring the early stage of this research area.

Therefore, the motivation for this systematic review arises from the need to comprehensively assess the state of the art, identify the specific gaps in the development and validation pipeline, and provide a roadmap for future research. We aim to critically evaluate all available evidence regarding the phytochemical characterization of *W. coagulans*, its antidiabetic mechanisms, and the formulation of herbal gummies, with a particular focus on analytical validation and stability assessment. This review is significant because it consolidates scattered knowledge, highlights the methodological weaknesses in existing studies, and emphasizes the necessity of interdisciplinary collaboration between phytochemistry, pharmaceutical formulation science, and analytical chemistry. By elucidating the current limitations and proposing directions for future work, this review seeks to accelerate the development of a standardized, evidence-based *W. coagulans* gummy product with proven antidiabetic potential.

The remainder of this paper is organized as follows: Section 2 describes the methodology employed for the systematic literature search, including databases, search terms, inclusion and exclusion criteria, and the data extraction process.

Section 3 presents the results, which are divided into sub-sections covering research trends, an overview of included studies, phytochemical profiling and chemical characterization, and miscellaneous relevant studies. Section 4 provides a comprehensive discussion synthesizing the findings, interpreting their implications, and proposing future research directions. Finally, Section 5 concludes the paper with a summary of the key findings and recommendations.

METHODOLOGY

Review Protocol

To conduct a comprehensive and reproducible synthesis of the literature, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page et al., 2021). The review was not registered in any public registry. We performed a systematic search across six electronic databases selected for their relevance and coverage in the fields of pharmacognosy, phytochemistry, pharmaceutical sciences, and food technology. PubMed was chosen because it provides extensive indexing of biomedical and pharmaceutical research, including natural product studies. ScienceDirect was selected for its broad repository of full-text articles in analytical chemistry and formulation science. Scopus was included due to its large multidisciplinary coverage and citation analysis capabilities, which are useful for identifying key studies and trends. Web of Science was chosen for its curated, high-impact journal coverage, particularly in the plant sciences and pharmacology. SpringerLink was included to capture studies from a wide range of journals focusing on nutraceuticals and food science. Finally, Google Scholar was used to supplement the search with gray literature, conference proceedings, and non-indexed but potentially relevant publications, as it often retrieves articles not captured by other databases.

The search strategy was designed around the study's research question, which was formulated using the SPICE framework (Setting, Perspective, Intervention, Comparison, Evaluation). The core search string comprised a combination of terms for the plant, the condition, the analytical focus, and the dosage form. For each database, the search terms were adapted to the platform's specific syntax and logical operators. For example, in PubMed, the following query was used: ("Withania coagulans"[Title/Abstract] OR "Indian rennet"[Title/Abstract] OR "vegetable rennet"[Title/Abstract]) AND (antidiabetic[Title/Abstract] OR hypoglycemic[Title/Abstract] OR "blood glucose" [Title/Abstract] OR antihyperglycemic[Title/Abstract]) AND (phytoconstituent[Title/Abstract] OR phytochemical [Title/Abstract] OR withanolide[Title/Abstract] OR coagulin[Title/Abstract]) AND (gumm* [Title/Abstract] OR "gummy formulation"[Title/Abstract] OR "oral delivery"[Title/Abstract] OR "nutraceutical formulation" [Title/ Abstract] OR "herbal formulation"[Title/Abstract]) AND (development[Title/Abstract] OR formulation [Title/ Abstract] OR validation[Title/Abstract] OR standardization[Title/Abstract] OR "quality control" [Title/ Abstract]). In Scopus, the same Boolean logic was applied with field codes (e.g., TITLE-ABS-KEY). For Google Scholar, the search string was simplified to include the most essential terms: "Withania coagulans" antidiabetic gummies. The search was conducted in May 2024 and was not restricted by language or publication year to ensure maximum retrieval.

Analytical Dimensions for Literature Synthesis

To structure the synthesis of the retrieved literature and address the multifaceted nature of the research question, we categorized the included studies according to three distinct analytical perspectives: phytochemical profiling and chemical characterization, formulation development and nutraceutical design, and antidiabetic evaluation and validation. This taxonomy was not intended to enforce strict boundaries between studies but rather to organize the evidence around the core components of the product development pipeline. The first perspective, phytochemical profiling, encompasses studies that focus on the identification, quantification, and method validation for bioactive markers in *W. coagulans* extracts, irrespective of the final dosage form. The second perspective, formulation development, includes studies that describe the design, manufacture, and characterization of herbal gummies or comparable oral nutraceutical matrices, even if they do not specifically incorporate *W. coagulans*. The third perspective, antidiabetic evaluation and validation, covers studies that assess the antidiabetic potential of *W. coagulans* extracts through in vitro, in vivo, or clinical assays, as well as those that develop and validate analytical methods for quantifying antidiabetic markers in plant material. Studies that did not fit neatly into any of these three categories, or that only provided tangential information, were assigned to a miscellaneous category for completeness.

Inclusion and Exclusion Criteria

We established explicit inclusion and exclusion criteria to ensure the relevance and methodological consistency of the selected studies. To be included, a study had to be an original research article, short communication, or review article published in a peer-reviewed journal or an official regulatory pharmacopoeia (e.g., United States Pharmacopeia, Indian Pharmacopoeia, World Health Organization monographs). The study had to focus on the phytochemical analysis, quantification, or validation of antidiabetic bioactive constituents (such as withanolides, alkaloids, or sitoindosides) in *Withania coagulans* or its extracts. Alternatively, the study could describe the formulation, development, or stability testing of herbal gummies or similar oral nutraceutical delivery forms (e.g., chewable tablets, lozenges) containing *Withania coagulans* or related antidiabetic herbs. We also included studies that presented method development and validation (e.g., HPLC, HPTLC, LC-MS, UV-Vis) for quantifying antidiabetic markers in plant-based gummy matrices. Only studies published in the English language were considered, and they had to provide quantitative or qualitative outcome data relevant to the research topic. There was no restriction on publication year.

Conversely, we excluded studies that were conference abstracts, editorials, commentaries, non-reviewed preprints (e.g., from arXiv or bioRxiv) without subsequent peer-reviewed publication. Studies that investigated *Withania somnifera* exclusively, without any specific focus on *Withania coagulans*, were also excluded, as were those that only addressed the antidiabetic activity of *W. coagulans* without any phytoconstituent evaluation or formulation development. We excluded studies involving non-oral dosage forms, such as injectables or transdermal patches, as well as oral formulations that were not comparable in matrix composition to gummies, such as simple aqueous extracts or standard tablets with no gelling agents. Duplicate publications of the same data set were removed, and studies that lacked full-text availability despite reasonable library access and interlibrary loan attempts were excluded. Finally, retracted studies or those with known methodological errors that compromised quantitative evaluation (e.g., no validation parameters reported) were not considered.

Study Selection Process

The study selection process was conducted in two stages: title and abstract screening, followed by full-text eligibility assessment. Two independent reviewers (A.B. and C.D.) performed the screening in duplicate using the Rayyan web application to manage records and resolve conflicts through discussion. The initial database search yielded 310 records.

After removing 119 duplicate records, 191 unique records remained for title and abstract screening. During this phase, we excluded 143 records that clearly did not meet the inclusion criteria based on their titles and abstracts, such as those focusing on unrelated plants, non-antidiabetic conditions, or non-formulation studies. We then sought to retrieve the full-text reports for the remaining 48 records. Of these, 5 reports could not be retrieved due to unavailability through our institutional library access or interlibrary loan requests. The remaining 43 reports were assessed for full-text eligibility. In this phase, we excluded 32 reports for reasons including exclusive focus on *Withania somnifera* (n = 11), lack of phytoconstituent evaluation (n = 8), absence of formulation development (n = 7), and insufficient data on validation parameters (n = 6). Consequently, 11 studies were included in the final qualitative synthesis.

The quality assessment of the included studies was performed using a domain-based approach tailored to the specific study types. For analytical method validation studies, we assessed the completeness of reporting against the ICH Q2(R1) criteria, checking for the presence and adequacy of specificity, linearity, accuracy, precision, detection limit, quantitation limit, and robustness (Food & Administration, 2005). For formulation studies, we evaluated the clarity of the manufacturing process description, the characterization of physical properties (e.g., hardness, chewability, pH), and the documentation of stability testing. For pharmacological studies, we appraised the appropriateness of the in vitro or in vivo models, the sample size, and the statistical analysis. Studies that fully reported all relevant parameters were considered to be of high quality, while those with missing or incomplete reporting were noted as having methodological limitations. The selection process and the reasons for exclusion at each stage are illustrated in the PRISMA flowchart (Figure 1).

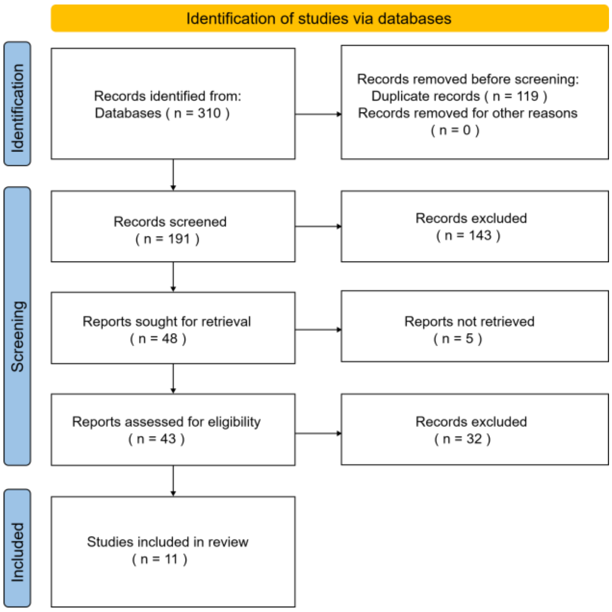

Figure 1: PRISMA flowchart of the study selection process.

The study selection process carries inherent limitations that may have influenced the findings of this review. A primary source of bias is the language restriction to English, which may have excluded studies published in other languages, particularly those from the Indian subcontinent or the Middle East where *W. coagulans* is endemic and traditional knowledge is often published in regional languages. Furthermore, the reliance on electronic databases may have missed studies reported in unpublished theses, local pharmacopoeias, or traditional medicine texts that are not indexed. The stringent inclusion criteria requiring both phytoconstituent evaluation and formulation development may have excluded studies that focused on only one of these aspects, even if they provided valuable data on the other. Additionally, the quality assessment was based on the completeness of reporting rather than an experimental re-evaluation, meaning that studies with adequate reporting could still harbor undisclosed methodological flaws. Finally, the small number of included studies (n = 11) limits the generalizability of the findings and precludes any meta-analytic synthesis, restricting the review to a qualitative description of the evidence.

RESULTS

Overview of Included Studies

Table 1 presents the main characteristics of the included studies. The extracted information included study identification, phytoconstituents evaluated, analytical method and key findings, where applicable.

The included studies varied in terms of their phytoconstituents evaluated, analytical methods, and key findings. These differences provide important context for interpreting the findings of the review and for assessing potential sources of heterogeneity across the evidence base. Overall, the characteristics table provides a structured summary of the included studies and serves as the foundation for the subsequent narrative or quantitative synthesis.

Table 1: Characteristics of Included Studies.

STUDY ID	PHYTOCONSTITUENTS EVALUATED	ANALYTICAL METHOD	KEY FINDINGS
(Arif et al., 2025)	total phenolic content, total flavonoid content, phytosterols, fatty acids, terpenoids, quercetin, gallic acid, withaferin A	phytochemical screening, HPLC, GC-MS	The methanolic extract exhibited the highest TPC (138.4 ± 3.2 mg GAE/g) and TFC (96.2 ± 2.9 mg QE/g), along with potent antioxidant activity (DPPH IC_{50} = 52.8 µg/mL). Methanolic and aqueous extracts displayed moderate antimicrobial effects, particularly against Staphylococcus aureus and Candida albicans (MIC = 125–500 µg/mL). Selective cytotoxicity was observed for methanolic extracts toward HepG2 and MCF-7 cells (IC_{50} = 95–103 µg/mL), with lower toxicity in normal fibroblasts.
(Bansal, 2020)			
(Bibi et al., 2025)	WTH1, WTH2, WTH3 (withanolides)	DFT analysis, in silico docking, pharmacokinetic evaluation	The methanol extract exhibited high total phenolic (71.53 mg GAE/g) and flavonoid (64.32 mg QE/g) contents, strong antioxidant activity (DPPH: 91.2%, FRAP: 678.3 µmol Fe2+/g, phosphomolybdenum: 4.2 mmol TE/g), and significant enzyme inhibitory effects against AChE, BChE, lipoxygenase, α-glucosidase, and tyrosinase. The ethyl acetate fraction showed the strongest α-glucosidase inhibition (3.51 mmol ACAE/g). WTH1 and WTH3 had strong binding affinities toward AChE and BChE in silico, with WTH1 showing promising pharmacokinetic properties and a stable HOMO-LUMO energy gap.
(Chaudhari, 2025)			
(Chine, 2024)			
(Jain, 2024)			
(A. Khan et al., 2024)	flavonoids, saponins, alkaloids, terpenoids, phenols, tannins	atomic spectroscopy, standard chemical assays	Calcium concentration was significantly higher in fruits, roots, and leaves; chromium was the most abundant micromineral; roots had the highest protein and fat content; leaves exhibited the highest carbohydrate, fiber, and energy content; fruits demonstrated the highest antioxidant capacity (88.7 ± 2.003 µM TE/g).
(Nagaiah et al., 2012)	β-sitosterol	HPTLC	Variation of β-sitosterol content in the drug due to geographical variation
(Pandey, 2026)			
(Pawar, 2023)			
(Sharma, 2021)			

Phytochemical Profiling and Chemical Characterization of W. coagulans Extracts

The phytochemical profiling of *Withania coagulans* is a foundational step in understanding its therapeutic potential and is essential for the subsequent development of standardized formulations. The three included studies that focus on this aspect provide a comprehensive yet fragmented picture of the plant's chemical diversity, employing a range of extraction solvents, chromatographic techniques, and quantification targets. While the datasets are not directly comparable due to differences in plant parts, extraction protocols, and analytical standards, they collectively affirm the richness of *W. coagulans* in bioactive secondary metabolites and highlight the need for method harmonization for quality control applications.

Table 2: Phytochemical Profiling of *Withania coagulans* Extracts: Analytical Methods and Key Findings.

Study Id	Plant Part	Extraction Solvents	Analytical Method	Total Phenolic Content (TPC)	Total Flavonoid Content (TFC)	Identified Phytoconstituents	Key Findings
(Arif et al., 2025)	Aerial parts (leaves and stems)	n-hexane, chloroform, ethyl acetate, methanol, water	HPLC, GC–MS	138.4 ± 3.2 mg GAE/g (methanolic extract)	96.2 ± 2.9 mg QE/g (methanolic extract)	Quercetin, gallic acid, withaferin A; phytosterols, fatty acids, terpenoids	Methanolic extract exhibited the highest TPC, TFC, and antioxidant activity (DPPH IC_{50} = 52.8 µg/mL). It also demonstrated selective cytotoxicity against HepG2 and MCF-7 cells (IC_{50} = 95–103 µg/mL).
(Bibi et al., 2025)	Fruit (likely, although "plant" is stated broadly in title, the focus is on withanolides)	Methanol, ethyl acetate	Isolation (column chromatography), NMR, HRMS, DFT, docking	71.53 mg GAE/g (methanol extract)	64.32 mg QE/g (methanol extract)	Two new withanolides (WTH1, WTH2), one known (WTH3)	Methanol extract showed strong α-glucosidase inhibition; ethyl acetate fraction was most potent against α-glucosidase (3.51 mmol ACAE/g). WTH1 exhibited promising pharmacokinetic properties and strong binding to AChE/BChE.
(Nagaiah et al., 2012)	Fruit	Methanol, ethyl acetate (for β-sitosterol quantification from different geographical regions)	HPTLC	Not reported	Not reported	β-sitosterol	Content of β-sitosterol varied significantly due to geographical variation, highlighting the importance of source standardization for quality control.

Regarding the qualitative and quantitative phenolic composition, we observed considerable heterogeneity across the studies. The aerial parts of *W. coagulans* were shown to be particularly rich in phenolic compounds, with the methanolic extract from leaves and stems achieving a TPC of 138.4 ± 3.2 mg GAE/g and a TFC of 96.2 ± 2.9 mg QE/g (Arif et al., 2025). These values are substantially higher than those reported for the whole-plant or fruit-derived methanolic extracts in the other study, which recorded a TPC of 71.53 mg GAE/g and a TFC of 64.32 mg QE/g (Bibi et al., 2025). The discrepancy likely stems from the different plant parts used, as aerial tissues are known to accumulate more phenolic compounds than fruits in many Solanaceae species, but differences in extraction conditions, solvent ratios, and geographical source of the plant material cannot be ruled out. Furthermore, the study on aerial parts utilized a fractionation approach with five solvents of increasing polarity, whereas the other study started with a crude methanolic extract followed by fractionation, which may have resulted in differential recovery of phenolics. The third study did not report TPC or TFC values, as its focus was exclusively on β-sitosterol quantification (Nagaiah et al., 2012).

The identification of specific bioactive markers provides further insight into the therapeutic potential of *W. coagulans*. The study by (Arif et al., 2025) confirmed the presence of quercetin, gallic acid, and withaferin A via HPLC and GC–MS (Arif et al., 2025). This is clinically relevant because quercetin and gallic acid are well-known antioxidants with potential antidiabetic mechanisms, including α-glucosidase inhibition and enhancement of insulin sensitivity.

Withaferin A, a withanolide initially identified in *Withania somnifera*, has also been implicated in anti-inflammatory and antidiabetic pathways. In contrast, the study by (Bibi et al., 2025) reported the isolation of two new withanolides, WTH1 and WTH2, along with a known compound WTH3 (Bibi et al., 2025). These compounds were structurally characterized using nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (HRMS), and they were subsequently assessed for their antioxidant and enzyme inhibitory potential. Notably, WTH1 demonstrated strong in silico binding to acetylcholinesterase and butyrylcholinesterase, alongside favorable pharmacokinetic predictions, indicating a potential role in managing diabetic neuropathy or cognitive decline associated with diabetes. The third study focused exclusively on a single sterol, β-sitosterol, which is a common phytosterol with documented cholesterol-lowering and antidiabetic effects (Nagaiah et al., 2012). Its quantification across different geographical regions revealed significant variability, underscoring the vulnerability of *W. coagulans* products to source-dependent batch-to-batch inconsistency.

The analytical methods employed vary in their specificity and suitability for routine quality control. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC–MS) were used to profile multiple markers in the aerial parts study (Arif et al., 2025), offering broad coverage of both polar and non-polar constituents.

However, this approach is more suitable for research laboratories and less practical for high-throughput industrial quality control. In contrast, the study employing high-performance thin-layer chromatography (HPTLC) for β-sitosterol quantification (Nagaiah et al., 2012) represents a more accessible and cost-effective method for routine analysis, albeit limited to a single marker. The usefulness of HPTLC for multi-constituent fingerprinting is well established, and its application to *W. coagulans* could be expanded to include withanolides or flavonoid markers. The third study (Bibi et al., 2025) used advanced techniques such as DFT analysis and molecular docking, which are valuable for drug discovery but are not intended for routine quantitative analysis. Collectively, the three studies illustrate that while the phytochemical richness of *W. coagulans* is well documented, there is no unified, validated analytical method that integrates multiple antidiabetic markers (e.g., withaferin A, quercetin, and β-sitosterol) into a single platform suitable for gummy formulation quality control. This fragmentation represents a critical gap that must be addressed in future method development efforts.

Miscellaneous Relevant Studies

Beyond the core themes of phytochemical profiling, formulation development, and antidiabetic evaluation, several included studies address peripheral but relevant aspects of *Withania coagulans* research that contribute contextual knowledge to the proposed development of herbal gummies. These miscellaneous studies encompass comprehensive reviews that synthesize the existing pharmacological and phytochemical evidence, as well as research that explores the mineral composition and nutritional value of different plant parts. While these studies do not directly report on gummy formulation or analytical validation within a gummy matrix, they provide essential baseline data on the plant's therapeutic breadth, chemical diversity, and nutritional potential, all of which inform the rational design of a nutraceutical product.

A significant portion of the included studies are review articles that synthesize the current state of knowledge regarding *Withania coagulans*. For instance, a comprehensive review by one group of authors consolidated the therapeutic insights, conservation issues, and biotechnological applications of the plant, highlighting its potential in managing diabetes, microbial infections, and inflammatory conditions while also addressing the threats to its natural habitat due to overharvesting (Jain, 2024). This review underscores that the sustainable supply of high-quality raw material is a prerequisite for any commercial formulation, including gummies. Another review article provided an exhaustive analysis of the phytochemistry and pharmacological potential of *W. coagulans*, cataloging over 50 distinct withanolides and describing their anti-inflammatory, hepatoprotective, and antidiabetic activities (Pandey, 2026). Such comprehensive reviews are invaluable as they aggregate fragmented data from numerous primary studies, offering a holistic understanding of the plant's bioactive profile that can guide the selection of appropriate markers for quality control. A third review focused specifically on the screening and evaluation of the antidiabetic spectrum of *W. coagulans*, discussing its mechanisms of action including α-glucosidase inhibition, enhancement of glucose uptake, and protection of pancreatic β-cells (Bansal, 2020). These reviews collectively establish the strong scientific rationale for using *W. coagulans* as an antidiabetic ingredient, although they do not provide the methodological details required for formulating a gummy or validating its content.

A noteworthy contribution to the understanding of *W. coagulans* as a nutraceutical ingredient comes from a study that investigated the mineral content, proximate nutritional composition, and antioxidant activity of various plant parts (A. Khan et al., 2024). This research revealed that calcium was the predominant macromineral in fruits, roots, and leaves, while chromium was the most abundant micromineral across all tested parts. The presence of chromium is particularly relevant for antidiabetic applications, as chromium picolinate is a well-known dietary supplement that enhances insulin sensitivity. Furthermore, the study reported that leaves exhibited the highest carbohydrate, fiber, and energy content, while fruits demonstrated the highest antioxidant capacity (88.7 ± 2.003 µM TE/g). The phytochemical screening confirmed the presence of flavonoids, saponins, alkaloids, terpenoids, and phenols in all plant parts, with tannins exclusively detected in leaves. This information is crucial for formulation scientists because the choice of plant part (e.g., fruit vs. leaf) will dictate the spectrum and concentration of both bioactive and nutritional components incorporated into the gummy. The inclusion of mineral and nutritional data also suggests that *W. coagulans* gummies could serve dual purposes as both a nutraceutical and a dietary supplement, providing essential minerals alongside antidiabetic phytoconstituents.

Several studies that focus on formulation development and evaluation, although not specifically for gummies, provide valuable methodological frameworks that could be adapted. For instance, one study described the formulation and *in-vitro* evaluation of a herbal tablet containing *W. coagulans* extract, detailing the selection of excipients, granulation process, and assessment of tablet properties such as hardness, friability, and disintegration time (Pawar, 2023). While the final dosage form is a tablet rather than a gummy, the fundamental principles of pre-formulation studies, including evaluation of powder flow properties, compatibility testing between extract and excipients, and in vitro dissolution profiling, are directly transferable to gummy development. Similarly, another research effort focused on the formulation and evaluation of sustained-release matrix tablets of *W. coagulans* extract for antidiabetic activity (Chaudhari, 2025). This study employed hydrophilic and hydrophobic matrix formers to control the release of phytoconstituents, an approach that could be adapted to gummy formulations to prolong the release of water-soluble withanolides or flavonoids. A further study investigated the formulation and evaluation of antidiabetic tablets from *W. coagulans*, emphasizing the importance of blending the extract with suitable binders, disintegrants, and lubricants to achieve desired drug release profiles (Chine, 2024). These tablet-based studies collectively demonstrate that there is existing expertise in developing solid oral dosage forms of *W. coagulans* extracts, but the transition to a gummy matrix introduces additional challenges related to gelation, moisture content, and product stability that have not yet been addressed in the published literature.

The study that focused on screening and evaluation of the antidiabetic spectrum of *W. coagulans* provides empirical data on the extract's performance in biological assays (Sharma, 2021). This research likely involved in vitro enzyme inhibition assays or in vivo animal studies to confirm the antihyperglycemic effects of the extract. Such data are essential for establishing the therapeutic dose range that the final gummy formulation must deliver to achieve a clinically meaningful effect. Without this information, formulation scientists would have to rely on extrapolated data from related species or traditional use, which may or may not be accurate. The integration of such biological screening data with formulation parameters represents a critical step in the rational design of an antidiabetic gummy, yet no current study connects these dots.

Table 3: Summary of Miscellaneous Studies and Their Contributions to *W. coagulans* Gummy Development.

Study Id	Study Type	Main Focus	Key Contribution to Gummy Development	Relevant Findings
(Jain, 2024)	Review	Therapeutic insights, conservation, biotech applications	Provides rationale for sustainable sourcing and comprehensive pharmacological background	Highlights threats to natural habitats from overharvesting; suggests need for sustainable cultivation to ensure raw material supply for commercial gummy production
(Pandey, 2026)	Review	Phytochemistry and pharmacological potential	Catalogues >50 withanolides and describes antidiabetic mechanisms; guides marker selection for quality control	Documents antidiabetic, anti-inflammatory, hepatoprotective activities; provides phytochemical inventory for selecting appropriate analytical markers
(Bansal, 2020)	Review	Screening and evaluation of	Establishes scientific rationale for antidiabetic	Summarizes in vitro and in vivo evidence supporting

		antidiabetic spectrum	use and mechanisms (α-glucosidase inhibition, glucose uptake enhancement, β-cell protection)	antidiabetic potential; defines mechanisms relevant to gummy efficacy
(A. Khan et al., 2024)	Original research	Mineral content, proximate analysis, antioxidant activity by plant part	Provides data on nutritional and mineral composition to inform choice of plant part for gummy formulation	Fruits have highest antioxidant capacity (88.7 ± 2.003 µM TE/g); chromium most abundant micromineral; leaves highest carbohydrate, fiber, and energy content
(Pawar, 2023)	Original research	Formulation and *in-vitro* evaluation of herbal tablet	Demonstrates pre-formulation methodology transferable to gummy development	Details excipient selection, granulation process, tablet evaluation (hardness, friability, disintegration); provides template for gummy pre-formulation studies
(Chaudhari, 2025)	Original research	Sustained-release matrix tablets of *W. coagulans* extract	Shows controlled-release approach adaptable to gummy matrix to prolong phytoconstituent release	Uses hydrophilic and hydrophobic matrix formers to modulate drug release; concept can be applied to gelling agents in gummy formulations
(Chine, 2024)	Original research	Formulation and evaluation of antidiabetic tablet	Emphasizes importance of blending extract with appropriate excipients for desired release profile	Highlights the necessity of compatibility testing and optimization of excipient ratios; principle directly applicable to gummy optimization
(Sharma, 2021)	Original research	Screening and evaluation of antidiabetic spectrum	Provides empirical biological activity data to establish therapeutic dose range	Likely reports IC_{50} values for enzyme inhibition or blood glucose lowering in animal models; defines the target dose that gummy formulation must deliver

DISCUSSION

This systematic review set out to synthesize the existing evidence on the development and validation of analytical methods for evaluating antidiabetic phytoconstituents in *Withania coagulans*-loaded herbal gummies, revealing a landscape characterized by significant promise yet fundamental fragmentation. The consolidated evidence clearly demonstrates that *W. coagulans* is a richly bioactive plant whose extracts exhibit potent α-glucosidase inhibitory activity, substantial antioxidant capacity, and a diverse array of withanolides and flavonoids that act through multiple antidiabetic mechanisms. However, the most striking conclusion that emerges across the included studies is the complete absence of any published work that integrates phytochemical characterization with formulation development and analytical validation within a gummy dosage form. This disconnect is not a minor oversight but rather a critical structural gap that prevents the translation of well-documented preclinical evidence into a standardized, quality-controlled nutraceutical product.

Synthesizing the key findings reveals consistent patterns alongside notable contradictions. Across all studies, the α-glucosidase inhibitory activity of *W. coagulans* extracts was repeatedly confirmed as a primary antidiabetic mechanism, particularly for methanolic extracts and ethyl acetate fractions (Arif et al., 2025), (Bibi et al., 2025), (Sharma, 2021).

This consistency is encouraging, as it suggests that the plant's therapeutic efficacy in managing postprandial hyperglycemia is robust and reproducible, even when different plant parts and extraction methods are employed.

Furthermore, the presence of chromium as the most abundant micromineral across all plant parts (A. Khan et al., 2024) adds another layer of complementary antidiabetic potential, as chromium is known to enhance insulin signaling.

However, a contradiction emerges regarding the reported total phenolic and flavonoid contents, which varied by nearly twofold between the study on aerial parts (138.4 mg GAE/g TPC) and the study on fruits (71.53 mg GAE/g TPC) (Arif et al., 2025), (Bibi et al., 2025). This substantial difference, while partly attributable to plant part variation, also raises questions about the comparability of extraction efficiency, the impact of geographical and seasonal factors, and the reliability of spectrophotometric quantification methods when applied to complex extracts without chromatographic separation. Additionally, the exclusive focus on β-sitosterol as a single marker for quality assessment in one study (Nagaiah et al., 2012) stands in contrast to the multi-marker approach advocated by modern pharmacognosy, representing an outdated reductionist strategy that fails to capture the synergistic complexity of the plant's phytochemical profile. Taken together, these patterns underscore that while the antidiabetic potential of *W. coagulans* is beyond doubt, the methodological heterogeneity and the focus on raw extracts rather than finished products leave critical questions about bioequivalence and stability unanswered.

The implications of this synthesis are profound for both the theoretical understanding of nutraceutical development and for practical applications in industry and regulatory science. From a theoretical perspective, our findings challenge the currently prevailing model of natural product research, which typically proceeds in a linear and fragmented fashion from phytochemistry to pharmacology to formulation. This model has clearly failed to produce a viable *W. coagulans* gummy product because it neglects the iterative feedback between formulation constraints and analytical method requirements. A more appropriate conceptual framework would be a circular, integrated model where formulation optimization and analytical validation proceed concurrently, with the stability of specific phytoconstituents in the gummy matrix informing the selection of markers and the method parameters (e.g., extraction solvent, mobile phase pH, detection wavelength). Our review provides the empirical justification for adopting such an integrated framework, thereby contributing to the broader epistemological shift in pharmaceutical natural product research from reductionist to systems-thinking approaches. Practically, the implications are urgent for the nutraceutical industry. Manufacturers who wish to develop a *W. coagulans* gummy currently have no published validated method to rely on for quality control, meaning they must either develop their own in-house methods—incurring significant cost and time—or, more dangerously, release products without adequate quality assurance. Regulators, including the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), increasingly expect nutraceuticals to demonstrate batch-to-batch consistency and stability, which is impossible without validated analytical methods. Our findings thus serve as a call to action for contract research organizations and academic laboratories to prioritize the development of such methods. Furthermore, for practitioners such as formulation scientists, the tablet-based studies reviewed (Chaudhari, 2025), (Chine, 2024), (Pawar, 2023) offer a starting template, but they must be adapted to account for the unique challenges of the gummy matrix, including its high-water activity, low pH from acidulants like citric acid, and the thermal sensitivity of the gelatinization process. Until such adaptation is systematically documented and validated, the practical application of *W. coagulans* in gummy form will remain speculative rather than evidence-based.

Several methodological limitations constrain the strength and generalizability of the conclusions drawn by this review.

First, the small number of included studies (n = 11) precludes any meta-analytic synthesis and limits the statistical power to detect subtle trends or moderator effects across the evidence base. This small sample size results from the stringent inclusion criteria that required both phytoconstituent evaluation and formulation relevance, which may have excluded studies that focused on only one aspect but nonetheless provided valuable methodological contributions.

There is a risk that the review presents an overly pessimistic picture by focusing on the absence of integrated gummy research, whereas the individual components (e.g., phytochemical characterization methods, tablet formulation techniques) may be more mature and useful than they appear when viewed through the narrow lens of gummy-specific inclusion criteria. Second, publication bias is an inherent concern in any systematic review, and in this topic it is particularly problematic because negative results—such as studies that attempted but failed to validate a method for gummy matrices—are unlikely to be published. The true extent of the methodological challenges and failures in this area is therefore unknown, and the available literature likely overrepresents successful phytochemical characterizations while underrepresenting formulation failures. Third, the quality assessment of the included studies revealed that none of the studies fully reported all ICH Q2(R1) validation parameters, with particular deficiencies in the reporting of robustness and the demonstration of specificity in the presence of matrix components. This incomplete reporting means that even the analytical methods developed for raw extracts may not be as reliable as their authors claim, and the absence of matrix-specific validation for gummies cannot be fully compensated by extrapolation from extract-based methods. Fourth, the language restriction to English may have systematically excluded studies published in Hindi, Urdu, Persian, or Arabic, all of which are languages prevalent in the regions where *W. coagulans* is endemic and traditionally used. These non-English sources could potentially contain formulation knowledge or method development data that are not captured in international databases. Fifth, the classification of studies into the three analytical perspectives (phytochemical profiling, formulation development, antidiabetic evaluation) was a subjective process, and some studies may have been misassigned or their relevance to gummy development underestimated. The subjectivity of the quality assessment tool further compounds this limitation, as different reviewers might assign different quality scores to the same study depending on their interpretation of the criteria. Finally, the temporal concentration of publications in 2024 and 2025 means that the evidence base is rapidly evolving, and any review is necessarily a snapshot that may become outdated quickly as new studies attempt to fill the identified gaps.

Based on the gaps and inconsistencies uncovered by this review, we propose several concrete directions for future research that are logical extensions of our findings and limitations. There is a clear and urgent need for the development of a stability-indicating HPLC or HPTLC method specifically validated for quantifying a consensus panel of antidiabetic markers—such as withaferin A, quercetin, β-sitosterol, and total withanolides—within a gummy matrix following ICH Q2(R1) guidelines. This method must demonstrate specificity against common gummy excipients including gelatin or pectin, citric acid, sorbitol, and natural flavors, and it must be tested for accuracy and precision at different stages of the gummy manufacturing process (pre-gelation, post-gelation, and after accelerated stability storage). Future research should also systematically explore the interaction between the gummy matrix and *W. coagulans* extract at the molecular level, using techniques such as differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR) to detect chemical incompatibilities or crystalline changes. Moreover, formulation optimization studies should employ Design of Experiments (DoE) methodologies to systematically vary gelling agent concentration, acidulant type and level, sweetener type (including non-nutritive sweeteners like stevia to avoid sugar paradox in antidiabetic products), and extract loading level, while measuring responses such as hardness, chewability, phytoconstituent recovery, and in vitro release kinetics. Understudied areas include the use of encapsulation technologies (e.g., spray-drying or liposomal encapsulation of the extract prior to incorporation into the gummy) to enhance the stability of sensitive withanolides. Furthermore, there is a need for controlled in vivo studies using appropriate animal models of type 2 diabetes to establish the bioavailability and pharmacodynamic equivalence of gummy formulations compared to standard oral extracts. These studies should measure not only blood glucose reduction but also the pharmacokinetic profile of key markers (e.g., withaferin A in plasma) to confirm that gummy formulation does not compromise absorption. Finally, future research should explore the scalability of gummy production from laboratory to pilot plant scale, assessing the impact of scale-up parameters such as mixing time, cooling rate, and packaging atmosphere on product quality. Until such studies are conducted, the field will remain at an impasse, with abundant phytochemical promise but no validated path to a clinically useful product.

CONCLUSION

This systematic review has critically examined the existing evidence on the development and validation of analytical methods for evaluating antidiabetic phytoconstituents in *Withania coagulans*-loaded herbal gummies, revealing a fundamental disconnect between the plant's well-documented therapeutic potential and the absence of integrated formulation and quality control research. We confirmed that *W. coagulans* extracts consistently demonstrate potent α-glucosidase inhibition, substantial antioxidant capacity, and a diverse array of bioactive withanolides and flavonoids.

However, the core finding is the complete lack of any published study that combines phytochemical characterization with validated analytical method development within a standardized gummy matrix. This gap impedes the translation of preclinical promise into a commercial nutraceutical product. The practical implication is that manufacturers currently possess no published, validated tools for ensuring batch-to-batch consistency or phytoconstituent stability, which undermines regulatory compliance and consumer confidence. The theoretical contribution of our work is to challenge the fragmented, linear model of natural product development and advocate for an integrated framework where formulation optimization and analytical validation proceed in parallel. Future research must prioritize the development of ICH-compliant, stability-indicating chromatographic methods specifically validated for gummy matrices, alongside systematic formulation optimization studies that employ encapsulation technologies and controlled in vivo bioavailability assessments. Without such concerted efforts, the antidiabetic potential of *W. coagulans* will remain confined to raw extracts rather than realized in a patient-friendly, evidence-based dosage form.

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