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Case Study

Data-Driven Pharmaceutical Development: Integrating Artificial Intelligence with Computational Modelling for Drug Synergy and Formulation Optimization

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ABSTRACT

Background: Artificial Intelligence (AI) assisted computational tools are advancing healthcare research and development. As drug discovery progresses toward development and clinical application, the adoption of these advanced computational tools is reshaping the landscape of pharmaceutical innovation. Two such tools: Response Surface Methodology (RSM) and Synergy Finder-have proven particularly valuable in optimising formulations and identifying effective drug combinations. **Objectives:** Two very specific case studies of each tool have been chosen to demonstrate the role of these tools in pharmaceutical innovation, highlighting real-world examples illustrating their practical use in developing neurotherapeutic nanoparticles and cancer-preventive therapy respectively. **Materials and Methods:** The selection of studies was guided by their widespread use in previously reported studies on brain-targeted delivery, nanoformulation optimisation and Synergy Finder based interaction evaluation. **Results:** In first case study, RSM combines experimental design with machine learning to simplify and optimize the brain targeting formulations to treat neurological disorders. In the second case, SynergyFinder aided in evaluating interaction (whether synergistic, additive, or antagonistic) among different compounds for cervical cancer prevention to identify the most suitable combinations. **Conclusion:** By integrating AI with pharmaceutical research through statistical modelling and bioinformatic analysis, these tools were found to support the development of more precise, optimized and efficient formulation strategies for safer therapies with lower toxicity and the potential to overcome resistance. This way, data-driven methodologies can streamline drug development and help to develop personalised, targeted therapies systematically.

Keywords: Artificial Intelligence, Computational Modelling, Drug Combination Therapy, Nanoparticle Drug Delivery, Response Surface Methodology (RSM), SynergyFinder.

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INTRODUCTION

An integrated artificial intelligence and computational modelling is revolutionizing the science and technology fields. Among its applications areas, advanced fields like drug discovery, materials science, and systems biology are quite interesting to study. There are various reasons of popularity of this integrated approach. Majorly, it includes its ability to process and interpret vast amounts of data, predictive capability, reduction in time and cost of research and development, handling of complex, multidimensional problems, automation and high-throughput analysis, personalisation and precision approaches, and interdisciplinary integration. In the present work we integrated this to predict outcomes, optimise formulations, and identify effective therapeutic strategies with reduced experimental burden in two applications, optimisation of drug delivery systems and evaluation of combination therapies. We utilized the first approach for neurological disorders and the second one for cancer.

In the first approach, to facilitates the systematic optimisation of formulation parameters through Design of Experiments (DoE), a statistical and computational tool called Response Surface Methodology (RSM) based studies are discussed. This works by modelling the relationships between independent variables and dependent variables (desired responses). We studied how RSM enabled efficient exploration of formulation space while minimising the number of experimental trials for the best possible management of neurological disorders in those conditions.[1,2]

In the second approach, in parallel, the growing interest in combination therapeutics has driven the development of computational platforms such as SynergyFinder, which quantitatively evaluated interactions between bioactive compounds. This tool applies multiple reference models,

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including Highest Single Agent (HSA), Bliss Independence, Loewe Additivity, and Zero Interaction Potency (ZIP), to classify interactions as synergistic, additive, or antagonistic across varying concentration ranges.[3] In this context, the present explores the previously published studies as case examples to illustrate the real time application of AI integrated computational tools in pharmaceutical research. Taken together, these case studies highlight how efficiently we may utilize formulation computational modelling followed by experimental validation to enhance the efficiency of drug optimization and facilitate the most efficient combination to design safer and more effective therapeutic strategies.[4]

MATERIALS AND METHODS

Materials

In optimized nanoparticle drug delivery systems, polymers and lipid-based excipients have been commonly employed, such as chitosan, PLGA and non-ionic surfactants (e.g., Tween 80®), were utilised for formulation development. The model therapeutic agents represented diverse physicochemical and pharmacological profiles, including neurotherapeutic (Rivastigmine, Minocycline, Quetiapine, 5-fluoro-2′-deoxyuridine, dopamine, etc.,) and antioxidant compounds (gallic acid, ellagic acid, resveratrol, rutin, curcumin etc.,) were selected to evaluate formulation performance. The selection of materials and drug candidates was guided by their widespread use in previously reported studies on brain-targeted delivery and nanoformulation optimisation.

In the second case study, plant materials (such as *Myrica esculenta, Vaccinium corymbosum, Rubus ellipticus*, and *Boerhavia diffusa*) were extracted using solvents (such as methanol) or their standard biologically active phytoconstituents (like quercetin, resveratrol, ellagic acid, benzoic acid, and rutin) were selected. The selection criteria is observed to be their antioxidant as well as anticancer property. It was observed that for these were then tested on cell line (like HeLa cell lines for cervical cancer) by seeding them in multi-well plates and treating them with different concentration range of the test biologically active phytoconstituents. The cells have been incubated followed by cell viability assessment by measuring their absorbance. The percentage inhibition was then calculated using IC_{50} value obtained from dose response curve of using MTT assay protocol and utilized further for dose matrix studies using various models.

Dataset Source

The previously published neurotherapeutic formulation and the published nutraceutical combination data are the dataset sources respectively for the two case studies. In the first case, the independent variables (polymer concentration, polymer drug ratio, surfactant concentration etc.,) as well as the responses (particle size, EE, activity etc.,) have been observed more specifically in the published literature. More specific steps involve RSM Model Development using the optimization designs, majorly CCD / Box Behnken design followed by model generation and the response prediction. After performing the optimization, response surface interpretation, predicted optimized formulation as well as comparison with published results were carried out. In nutshell, such a systematic optimization analysis led to a reduction in experimental runs and improves prediction within the design space which help take the decisions related to the optimized formulation development in this selected case.

In the second case study, the major data source is the dose matrix preparation observed from laboratory setup condition. These were subjected to the Synergy Finder Analysis using various models like, Bliss model, Loewe model and ZIP synergy model. The next step is Synergy Mapping where heatmap interpretation and identification of synergistic combinations is performed. This study majorly signifies combination prioritization and predicts the dose reduction potential.

METHODOLOGY AND STUDY DESIGN

The study aims to demonstrate the application of artificial intelligence-assisted tools in pharmaceutical research. For that, the computational-experimental approach of the present work adopted previously validated methodologies reported in the literature. We considered two case studies First, the optimisation of brain-targeted nanoparticle formulations using Design of Experiments (DoE)-based Response Surface Methodology (RSM) which is schematically represented in Figure 1. The second one involve the evaluation of anticancer bioactive combinations using a synergy analysis platform SYNERGYFINDER and its generalized process is graphically represented in Figure 2.

Case Study I: DoE-Based Optimisation of Nanoparticle Formulations for Neurological Disorders

The restrictive blood-brain barrier and complex CNS pathology are very important mechanism to maintain homeostasis by blocking 100% large molecules and 98% of small molecules across the brain. So, for maintaining this, even the therapeutic compounds are unable to cross BBB. Researchers have tried diverse approaches (invasive as well as non-invasive) and among the non-invasive one, the formulation types (chitosan nanoparticles, lipid-based carriers, nanoemulsions, and liposomes, etc.,) for favourable delivery of therapeutic actives to brain in stable form. Among these, the nanocarriers based approach is the most widely adopted. But it needs specific parameters viz., size of the carrier, its stability and entrapment efficiency, etc. to be achieved for a favourable delivery.[5] These all responses are dependent on many independent variables to be controlled viz., the concentration of polymer, the time of agitation to make it, the sustained or controlled drug release, temperature etc. Interestingly, to make the most effective formulation, One Factor at A Time (OFAT)

approach can be used where one variable will be changed and its effect on the responses would be monitored. But, if we use that approach, the number of experimental runs would be so high. Also, how one independent variable behave in presence of the other variable (interaction terms) cannot be predicted.

Based on the published studies, we observed a consistent trend across the compiled studies which is Design of Experiment (DoE) based Response Surface Methodology (RSM). It has been reported to be the reliable strategy where there is a clear shift toward using predictive polynomial models. These predictive models are obtained by integrating computational optimisation with experimental validation. In this approach, there is not only reduced experimental variability, but also strengthening of confidence in the optimisation process. In most of the cases, Central Composite Design (CCD) and Box-Behnken Design (BBD) have been utilized very efficiently. It is important to note that these designs also utilize the interaction terms among the variables and are capable to capture the nonlinear relationships between formulation variables and critical quality attributes.[6] Such AI-assisted response surface-based nanoformulation optimisation practice favors a systematic and precise control of independent variables which govern brain uptake and therapeutic efficacy of the encapsulated active moiety. Across the different formulation types, polymeric nanoparticles (like chitosan and PLGA nanoparticles), lipid-based carriers, nanoemulsions, and liposomes have been optimized. The target of performing the optimisation is primarily directed toward controlling the particle size, enhancing drug entrapment efficiency, and achieving favourable zeta potential, all of which are crucial for effective blood-brain barrier penetration and stability. Collectively, these studies demonstrate that DoE-driven approaches not only streamline formulation development but also enable rational, data-driven systematic design, where are routinely validated through *in vitro* laboratory studies as well as pre-clinical behavioural and biochemical outcomes experimentally.[7-11]

Case Study II: SynergyFinder-Based Evaluation of Anticancer Bioactive Combinations

The major challenge in oncology clinically is the adverse effects of drugs when exposed at anticancer dose for cancer treatment. The Synergy Finder based studies reveals a very systematic approach to evaluate drug and bioactive combinations which are based on integration of AI with computation. The complex response relationships have been evaluated through established reference models like HAS, Bliss, Loewe and ZIP. These models support the development of a reproducible therapeutic design which makes us understand the interaction across concentration ranges.[12] The comparative summary of the various rreference models for drug combination synergy analysis is shown in Table 1.

This work focuses on studying those reports where synergy analysis has been utilized to bioactive phytoconstituents which have shown antioxidant and anticancer effects. A few such bioactive phytoconstituents' examples are Quercetin, resveratrol, rutin etc. But, when used individually, their efficacy is not that significant as being limited many factors such as low potency, lower bioavailability, and inconsistent cellular responses. However, the mechanistic synergy, especially with complimentary mechanism of action, is very well explored quantitatively using this software tool.[13]

These combination tool help find out the best possible combination ratio in quantitative manner at which comparatively superior efficacy is achieved within the available resources. This is a very good outcome and may even lead to a reduction in dose across the possible ratio combinations and that too with lesser toxicity. Such a systematic tool helps in deeper understanding the pattern of these interactions across concentration ranges. This way, the approach can support more rational and reproducible therapeutic design as many a times, it is possible that a lower concentration of individual compounds but in combination (synergism) can achieve comparable or superior cytotoxic effects relative to higher doses of single agents. Once the dose of both drugs which are being combined will be used, this will be an attempt to minimize the adverse effect as well as to overcome the drug resistance mechanisms too.[14,15]

Using SynergyFinder tool, one can systematically quantify dose-dependent bioactive interactions by interpreting dose-response matrices. When the multiple reference models are used for such analysis, the response become more reliable and we can say they are validated as consistent trends are observed across HSA, Bliss, Loewe, and ZIP analyses. Such platform can facilitate a comprehensive assessment of interaction profiles. Not limited to predict synergism, the additive as well as antagonistic effects can also be predicted using such a systematic approach.[16]

To illustrate the practical value of this tool, two studies are discussed here which assessed phytochemical combinations against HeLa cervical cancer cells. When analysed using different models, **dose-dependent synergistic interactions**, with certain combinations showing enhanced cytotoxicity compared to individual compounds.

To demonstrate the practical applicability of this approach, previously reported studies evaluating phytochemical combinations against HeLa cervical cancer cell lines were analyzed. Cervical cancer remains a significant global health burden, primarily associated with persistent infection by high-risk Human Papillomavirus (HPV) strains, and continues to pose challenges due to limitations in current treatment modalities, including toxicity and accessibility.[16,17] Among the investigated combinations, quercetin and ellagic acid have been widely studied due to their complementary anticancer mechanisms. Quercetin exhibits antioxidant, anti-proliferative, and pro-apoptotic effects by modulating signaling pathways

involved in cell cycle regulation and apoptosis.[14,18] Ellagic acid has been reported to induce cell cycle arrest and upregulate tumor suppressor genes such as p53 and p21, thereby inhibiting cancer cell proliferation.[19] The combination of these phytoconstituents demonstrated significantly enhanced anticancer activity compared to individual treatments. Specifically, the combination resulted in substantial reduction in cell viability, with reported inhibition values approaching ~88%, along with high synergy scores (e.g., HSA score >30), indicating strong synergistic interaction.[13,20] These findings were further supported by computational modelling using SynergyFinder 3.0, confirming the presence of dose-dependent synergistic regions within the tested concentration range. Overall, these results highlight the potential of AI-assisted synergy analysis platforms in identifying effective combination strategies. By integrating computational modelling with experimental validation, this approach enables the rational design of multi-component therapies with improved efficacy, reduced toxicity, and enhanced translational potential in cancer treatment.[21]

Statistical Analysis

Data were systematically evaluated using mathematical modeling. For formulation optimization, quadratic polynomial models were developed, and statistical validation was conducted via Analysis of Variance (ANOVA), utilizing F-values, *p*-values (<0.05), and coefficient of determination (R^2) for adequacy. For evaluating combinations, dose-response matrices were analyzed using multiple computational reference models, including Highest Single Agent (HSA), Bliss Independence, Loewe Additivity, and Zero Interaction Potency (ZIP), to generate synergy scores.

Ethical Statement

This case study did not involve any animal or human subjects.

RESULTS

Overview of Case Study-Based Analysis

To evaluate the role of selected two AI tools, we overviewed the findings from previously reported experimental and

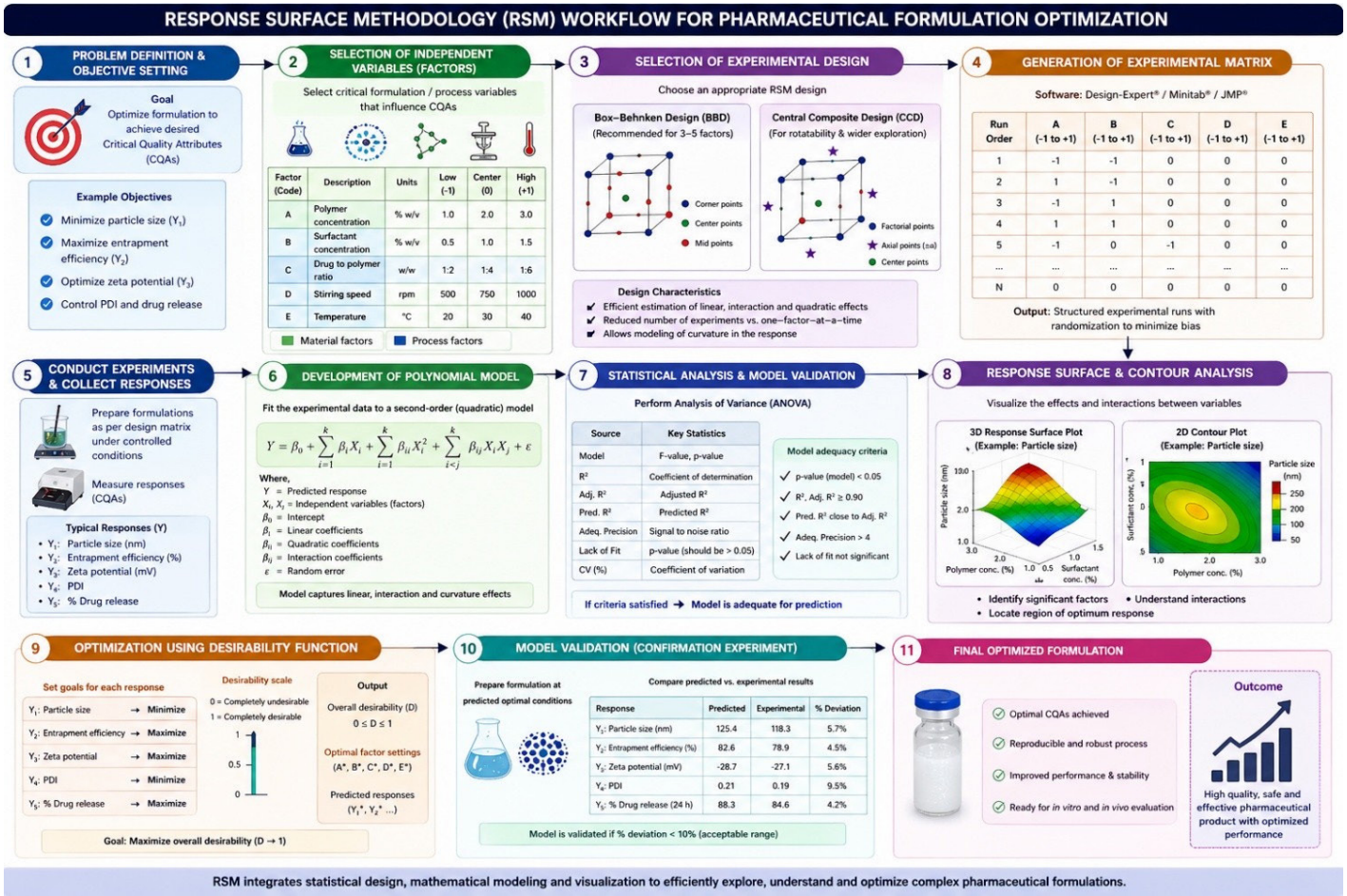


Figure 1: Advanced schematic representation of Response Surface Methodology (RSM)-driven optimization workflow for pharmaceutical formulation development. The workflow illustrates a systematic progression from problem definition and selection of critical formulation variables through experimental design (Box-Behnken/Central Composite Design), generation of experimental matrix, and execution of controlled experiments. It further depicts the development of quadratic polynomial models, statistical validation using ANOVA, and visualization via response surface and contour plots to elucidate variable interactions. Optimization is achieved through desirability function analysis, followed by model validation and confirmation experiments, culminating in the identification of a robust, reproducible, and optimized formulation with enhanced Critical Quality Attributes (CQAs).

Table 1: Comparative Evaluation of Reference Models for Drug Combination Synergy Analysis.[13,14]

Model	Conceptual Framework	Mathematical Basis	Core Assumption	Definition of Additivity / Null Model	Synergy Interpretation	Applicability	Strengths	Limitations
HSA (Highest Single Agent)	Non-interaction reference comparing combination response to the most effective single agent.	EHSA=max(EA ,EB)	No interaction; combination effect should not exceed the best single agent.	Additivity is defined as the maximum effect produced by either drug alone.	Synergy when observed combination effect > (E_{HSA}); antagonism when < (E_{HSA}).	Preliminary screening; high-throughput datasets	Simple, intuitive, minimal computation; no model fitting required.	Ignores interaction mechanisms; underestimates synergy in many cases.
Bliss Independence	Probabilistic model assuming independent drug actions.	EBliss=EA+EB−(EA·EB)	Drugs act independently through distinct biological pathways	Additivity is defined in terms of probabilistic independence of effects.	Synergy is when observed effect > expected Bliss value.	Suitable for those drugs which have different mechanisms of action.	Mechanistically meaningful for independent pathways; widely applicable.	Overestimates synergy when drugs share pathways; ignores dose equivalence.
Loewe Additivity	Dose equivalence principle assuming similar mechanism of action.	$(d_A/D_A)+(d_B/D_B)=1$	Drugs are dilutions of each other (similar targets/ mechanisms).	Additivity defined by equivalent dose substitution.	Synergy when combined doses required are less than additive expectation.	Ideal for drugs targeting the same receptor/pathway.	Considered gold standard for similar-acting drugs; mechanistically rigorous.	Computationally complex; not valid for independent mechanisms.
ZIP (Zero Interaction Potency)	Hybrid model integrating Bliss independence and Loewe additivity.	Compares deviation in both response and potency landscapes.	No change in potency or efficacy upon combination	Additivity defined by no interaction in dose-response surface.	Positive ZIP score → synergy; negative → antagonism; zero → additive effect.	General-purpose model for diverse drug combinations.	Robust, integrates multiple interaction dimensions; widely used.	Computationally intensive; interpretation requires expertise.

computational studies. In the two domains: (i) DoE-driven optimisation of brain-targeted nanoparticle formulations and (ii) synergy-based evaluation of anticancer bioactive combinations. The results are not primary experimental observations but are a comparative analysis and opinion sharing of the reported observations.

DoE-Based Optimisation of Brain-Targeted Nanoparticle Systems

When primary experimental datasets from published studies were evaluated, the findings consistently demonstrated that the application of Design of Experiments (DoE) coupled with Response Surface Methodology (RSM) enabled efficient statistical optimization of nanoparticle formulations intended for brain delivery. Across multiple studies, nanoformulations were predominantly developed using polymers such as chitosan, Poly (Lactic-Co-Glycolic Acid) (PLGA), and Polybutyl Cyanoacrylate (PBCA), as well as lipid-based systems including Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs). These systems were optimized by varying critical formulation variables such as polymer concentration (0.5-3% w/v), surfactant concentration (0.2-2% w/v), and drug-to-polymer ratio (1:1 to 1:5), which significantly influenced key critical quality attributes. Table 2 summarize some studied to describe how Central Composite Design based Response Surface Methodology has been applied to neurotherapeutic nanoparticle systems. RSM analysis across these studies revealed pronounced nonlinear relationships between independent variables (formulation factors) and dependent variables (responses), including particle size, Entrapment Efficiency (EE), Polydispersity Index (PDI), and zeta potential. Typically, optimized formulations achieved particle sizes in the range of 80-180 nm, entrapment efficiencies of 70-92%, and zeta potential values between ±20 to ±35 mV, indicating good stability and suitability for brain targeting. In several studies, increasing polymer concentration resulted in an increase in particle size (by ~20-40%), while higher surfactant levels contributed to size reduction and improved dispersion stability.[9,10]

The polynomial regression models generated through RSM exhibited strong predictive capability, with coefficient of determination (R^2) values ranging from 0.92 to 0.99, adjusted R^2 values above 0.90, and non-significant lack-of-fit ($p>0.05$) in most cases. The agreement between predicted and experimentally observed responses was consistently high, with percentage deviation typically below 5-10%, confirming the robustness and validity of the developed models. Graphical visualization through two-dimensional contour plots and three-dimensional response

surface plots further illustrated the interaction effects between formulation variables. These plots clearly demonstrated that the relationship between factors and responses was predominantly nonlinear, with significant interaction terms ($p<0.05$) indicating combined effects of variables on formulation performance. A typical representation of the three-dimensional response surface plot and corresponding two-dimensional contour plot illustrating the effect of formulation variables on particle size during Response Surface Methodology (RSM)-based optimization is shown in Figure 3. Unlike the conventional One-Factor-at-A-Time (OFAT) approach, RSM enabled simultaneous evaluation of multiple variables and their interactions, significantly improving the efficiency of the optimization process. Another notable advantage of RSM is its ability to predict optimal formulation conditions with high precision. The desirability function approach allowed identification of optimal factor levels that simultaneously satisfied multiple response criteria. In most studies, optimized formulations achieved a 20-50% reduction in particle size and a 15-30% increase in entrapment efficiency compared to non-optimized formulations.[11,22]

Furthermore, optimized nanoformulations demonstrated improved biological performance. *In vitro* drug release studies indicated controlled and sustained release profiles (up to 24-48 hr), while *in vivo* and behavioral studies reported enhanced therapeutic efficacy, including improved cognitive performance and increased brain drug uptake.[23] These findings confirm the functional relevance of RSM-optimized formulations in neurotherapeutic applications. Overall, statistically significant models with high F-values ($p<0.05$), strong goodness-of-fit ($R^2>0.9$), and non-significant lack-of-fit validated the predictive capability of the RSM approach. The use of Design-Expert® software further facilitated efficient design space exploration and optimization. Compared to traditional OFAT methods, RSM reduced the number of experimental runs by approximately 40-70%, while providing more comprehensive and reliable insights into formulation behavior. These results highlight the effectiveness of RSM as a data-driven tool for rational and efficient development of nanoparticle-based drug delivery systems.[24,25]

Case Study II-SynergyFinder for Anti-Cancer Active compounds

Dataset Source

Previously published experimental datasets involving anticancer bioactive combinations were utilized as the primary source for synergy analysis. The selected case study focused on phytochemical compounds, particularly polyphenols such as resveratrol and ellagic acid, which have been extensively investigated for their anticancer potential. These compounds were evaluated both individually and in combination across a range of concentrations using HeLa cervical cancer cell lines. The biological response was quantified in terms of percentage cell viability/inhibition, obtained through standard cytotoxicity assays (e.g., MTT assay), forming the basis for dose-response analysis.[26,27]

To facilitate computational evaluation, the experimental data were systematically organized into a structured dose-response matrix. In this matrix, increasing concentrations of the two bioactives were arranged along the rows and columns, while each cell represented the observed biological response corresponding to a specific concentration pair. This structured format is essential for accurate interpretation of interaction effects, as it enables comprehensive analysis of dose-dependent behavior across multiple combination points. Although such matrices can be extended to higher-order combinations, the present study focuses on binary combinations for clarity and interpretability. The prepared dose-response matrix was subsequently analyzed using the SynergyFinder 3.0 platform. Interaction analysis was performed using established reference models, particularly the Highest Single Agent (HSA) model, to quantify the nature of interactions as synergistic, additive, or antagonistic. The platform computes numerical synergy scores across the entire dose matrix

Table 2: Summary of Central Composite Design-based Response Surface Methodology applied to neurotherapeutic nanoparticle systems, employing a two-level, three-factor CCD to highlight optimised formulation attributes and validated *in vivo* outcomes.

Sl. No.	Study / Drug System	Disease Model	Key Optimized Responses	Predicted Value (From RSM)	Observed Value (Experimental)	Prediction Error (%)	Major Biological Impact
1.	Minocycline HCl NPs (cMHNP)9	Neurodegeneration / Memory impairment	↓ Particle size, ↑ %DEE	150 nm; 90%	161.57±12.86 nm; 89.63±2.66%	≤8%	~9-fold ↑ brain drug level; improved EPM & MWM performance
2.	Gallic Acid NPs (cGANP)10	Anxiety-like disorder	↓ Particle size, ↑ %DEE	105.65 nm; 95.40%	103.33±2.28 nm; 93.62%	<3%	Significant anxiolytic activity; ↓ plasma nitrite
3.	Rivastigmine NPs (cRTNP)11	Alzheimer's / Amnesia	↓ Particle size, ↑ ZP, ↑ %DEE	~150 nm; >+20 mV; >94%	154.06±2.14 nm; 26.6±0.9 mV; 96.36±1.09%	≤3%	Reversal of scopolamine-induced amnesia; ↑ MTD by 10%

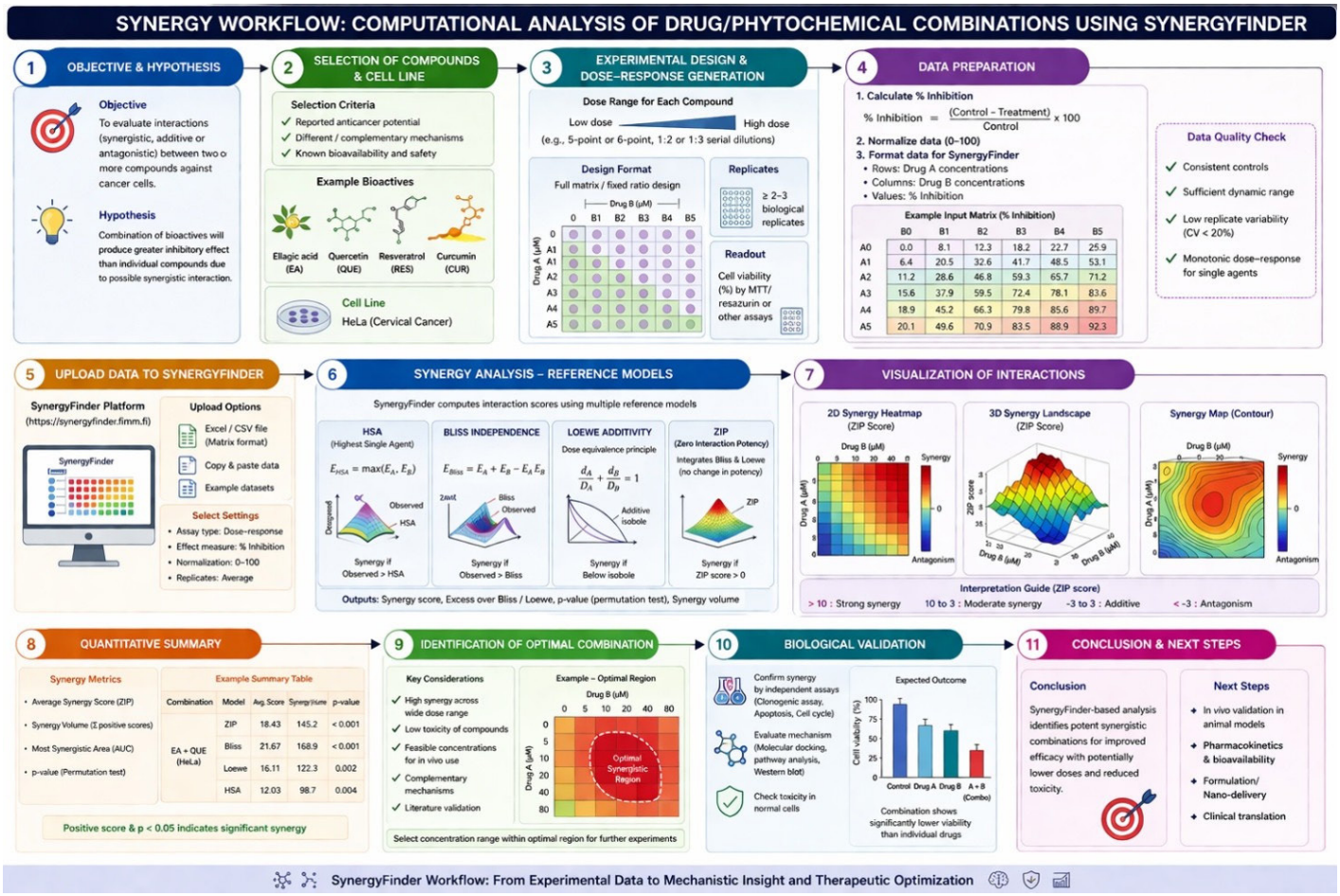


Figure 2: Comprehensive workflow of SynergyFinder-based computational analysis for evaluating drug/phytochemical combinations. The schematic illustrates the sequential process from objective definition and selection of bioactive compounds and cell models, through experimental design and dose-response matrix generation, to data normalization and quality control. It further depicts the application of multiple reference models (HSA, Bliss independence, Loewe additivity, and ZIP) for quantitative synergy scoring, followed by visualization using heatmaps, dose-response curves, and three-dimensional interaction landscapes. The workflow culminates in ranking and selection of optimal synergistic combinations, supported by biological validation and mechanistic studies, enabling rational design of combination therapies with enhanced efficacy, reduced toxicity, and improved translational potential.

and generates visual representations such as heatmaps and three-dimensional interaction landscapes, providing a detailed understanding of interaction dynamics.[28]

The results demonstrated a clear dose-dependent increase in cytotoxicity with increasing concentrations of both resveratrol and ellagic acid. The average HSA synergy score across all tested combinations was 18.26, indicating a moderate but consistent synergistic interaction. Notably, a specific concentration pair-2 μg/mL of resveratrol combined with 1.5 μg/mL of ellagic acid-exhibited the most pronounced effect, with a maximum synergy score of 42.1 and complete cytotoxic response (100% cell death) (Table 3). Additionally, the combination index (CI < 1) further confirmed true pharmacodynamic synergy. Visualization through synergy heatmaps revealed that regions corresponding to mid-to-high concentration ranges exhibited strong positive synergy scores (represented by red/yellow gradients), indicating enhanced combined efficacy compared to individual treatments. In contrast, lower concentration regions predominantly showed additive or mildly antagonistic interactions (blue regions), highlighting the concentration-dependent nature of the interaction. These findings confirm that optimal therapeutic outcomes are highly dependent on precise dose selection. Comparative analysis across different models further indicated that the HSA, Bliss, and ZIP models consistently identified similar regions of synergy, reinforcing the robustness of the observed interaction patterns. The ZIP and Bliss models, in particular, demonstrated high sensitivity in detecting synergistic regions, supporting their applicability in combination screening studies.[20,21]

Mechanistically, the observed synergy between resveratrol and ellagic acid can be attributed to their complementary modes of action. Figure 4 represent SynergyFinder based integrated multi-panel approach of dose-response behavior and synergy analysis of resveratrol and ellagic acid combinations. Resveratrol is known to modulate key signaling pathways such as NF-κB, PI3K/Akt, and MAPK, while ellagic acid induces apoptosis through p53 activation and Reactive Oxygen Species (ROS) generation. Additionally, resveratrol may enhance cellular uptake

and membrane permeability, thereby facilitating the intracellular activity of ellagic acid. This complementary interaction results in amplified anticancer effects when both compounds are used in combination. Overall, the integration of experimental dose-response data with AI-assisted computational analysis provides a robust framework for identifying optimal combination strategies. The findings demonstrate that synergistic combinations can achieve enhanced cytotoxic efficacy at lower individual doses, thereby reducing potential toxicity and improving therapeutic outcomes. This approach holds significant potential for the rational design of combination-based nutraceutical formulations and advanced drug delivery systems for cancer therapy.[28-30]

DISCUSSION

A comparative evaluation of both case studies highlights a common underlying trend: the transition from empirical experimentation to data-driven optimisation frameworks. While SynergyFinder facilitates the identification of effective drug combinations through systematic interaction analysis, RSM enables efficient optimisation of formulation parameters with minimal experimental runs.

Both approaches demonstrate the ability to capture complex, multidimensional relationships, whether in the context of drug-drug interactions or formulation variable interplay. The analysed studies collectively indicate that integrating computational tools with experimental workflows enhances precision, reduces uncertainty, and improves the efficiency of pharmaceutical development processes.

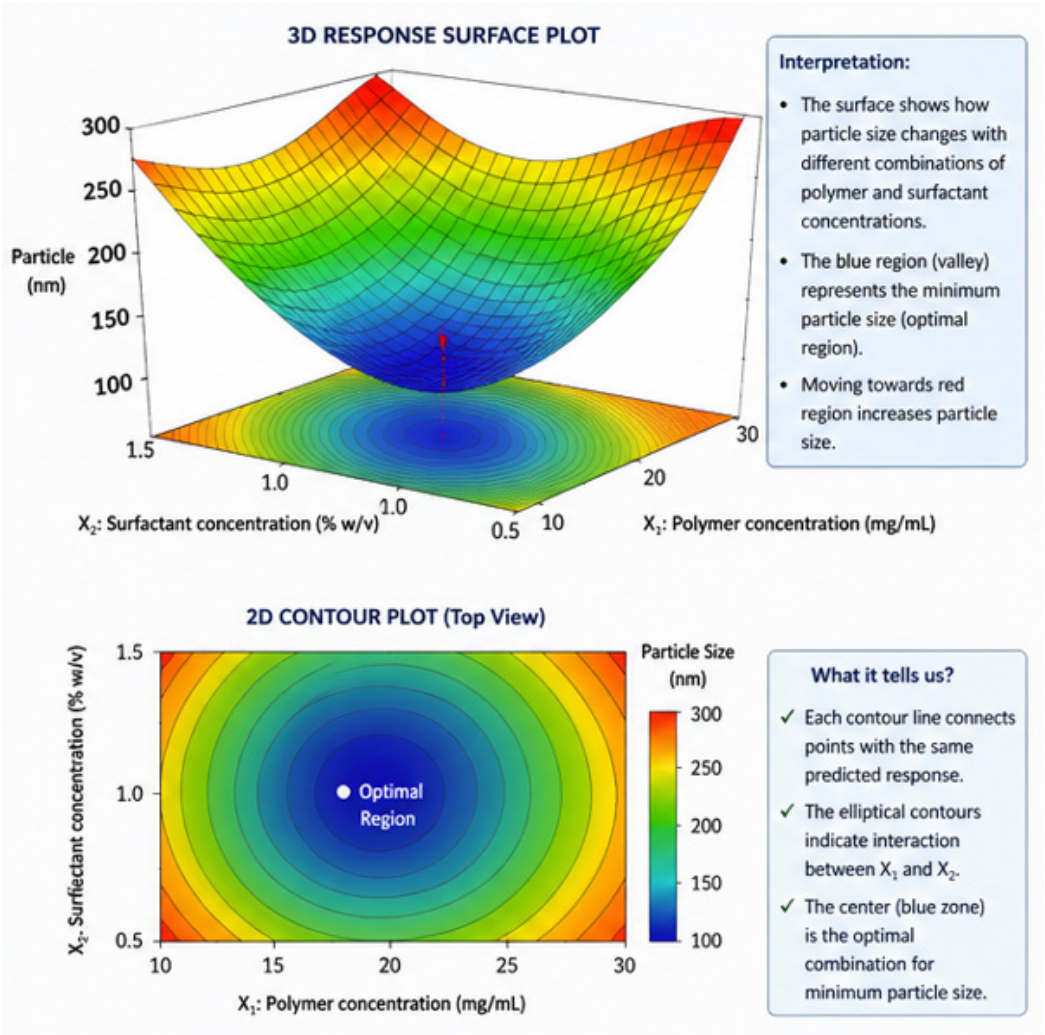


Figure 3: Three-dimensional response surface plot and corresponding two-dimensional contour plot illustrating the effect of formulation variables on particle size during Response Surface Methodology (RSM)-based optimization. The 3D surface represents the combined influence of polymer concentration (X_1) and surfactant concentration (X_2) on the response (particle size), highlighting a nonlinear relationship between variables. The contour plot provides a top-view visualization where concentric color gradients indicate response magnitude, with the central blue region representing the optimal formulation condition corresponding to minimum particle size. The elliptical contour pattern further confirms significant interaction between the independent variables, enabling precise identification of optimal design space for formulation development.

Table 3: Summary of SynergyFinder-based HSA modelling outcomes showing optimal dose combinations, synergy strength (HSA and CI), and cytotoxic responses of binary and ternary bioactive compound combinations evaluated on HeLa cervical cancer cells.[20,21]

Combination	Optimal Dose (µg/mL)	Max HSA (CI)	Cytotoxicity (%)	Key Insight
Quercetin + Resveratrol	2 + 0.5	50.1 (0.47)	68	Highest synergy score
	2 + 2	32.4 (0.42)	90	Best biological effect
Quercetin + Ellagic acid	4 + 1.5	31.2 (0.64)	88	Narrow synergy window
Resveratrol + Ellagic acid	2 + 1.5	42.1	100	Strong binary synergy
Quercetin + Ellagic acid + Resveratrol	4 + 1.5 + 0.5	42.7	100	Multimodel-confirmed
Benzoic acid + Rutin	1.2 + 1.2 (mg/mL)	29 (0.64)	92	CSS-validated synergy

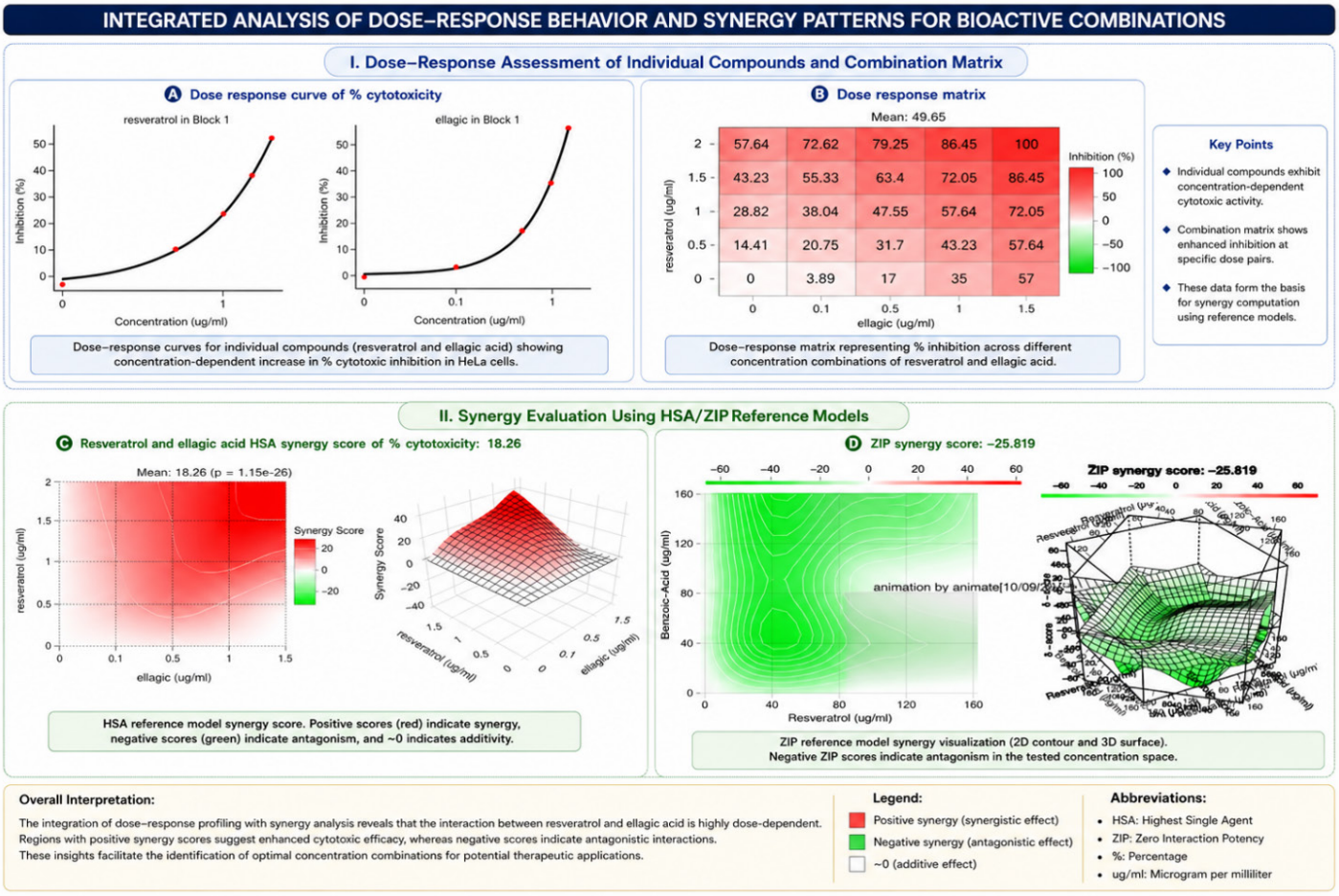


Figure 4: Integrated multi-panel representation of dose-response behavior and synergy analysis of resveratrol and ellagic acid combinations evaluated using SynergyFinder. (A) Dose-response curves of individual compounds demonstrating a concentration-dependent increase in cytotoxic inhibition, establishing baseline pharmacological activity. (B) Dose-response matrix depicting percentage inhibition across varying concentration combinations, serving as the primary dataset for interaction analysis. (C) Synergy visualization based on the HSA reference model, presented as a two-dimensional contour map and three-dimensional surface plot, where positive scores (red regions) indicate synergistic interactions and values near zero indicate additive effects.(D) Synergy assessment using the ZIP model, illustrated through contour and surface representations, highlighting predominantly negative interaction scores indicative of antagonistic behavior across the evaluated concentration range.Overall, the integrated analysis demonstrates that interaction effects between the bioactives are strongly dose-dependent, with distinct regions of synergism and antagonism, thereby emphasizing the importance of precise concentration optimization in combination-based therapeutic strategies.

The present case studies utilizing Design Expert and Synergy finder highlights the growing relevance of artificial intelligence-enabled tools' utilization in pharmaceutical sciences. They not only simplify complex optimization challenges but also can help in taking informed decisions which are very useful in pharmaceutical and nutraceutical research. The Response Surface Methodology (RSM) promises an effective and systematic assessment of multiple formulation variables and can identify optimal conditions with limited sets of experiments within the design space (available resources and condition set). The best part is utilizing the interaction effects and nonlinear relationships which RSM provide which helped in refining neurotherapeutic formulations. Similarly, the use of SynergyFinder offered valuable insights into the interaction dynamics of multi-component anticancer bioactive combinations. Utilizing this tool, we may quantify and clearly differentiate between synergistic, additive, and antagonistic effects across varying dose ranges. It opens the possibility of rational design of combination therapies, particularly in anticancer applications where multi-target approaches are often required. Importantly, the present work relies on previously published datasets, yet generates new analytical interpretations through computational modeling, thereby reinforcing its research-oriented contribution rather than a descriptive review. The digital images of various reports independently demonstrate both tools to understand practical utilization of them in real time problem solving and addressing distinct formulation development challenges. Overall, the findings support the broader applicability of AI-assisted methodologies in neurological and anticancer development, offering a systematic, data-driven, efficient strategy for optimizing both single formulations and combination-based therapeutic systems in other applications as well.

CONCLUSION

The case studies presented here represent how the AI-assisted tools can address key challenges in formulation optimization and combination analysis. The first case study revealed that Design Expert can enabled systematic evaluation and optimization for neurotherapeutic formulation variables with improved quantitative prediction capability with minimum experimental runs. The second case study based on the SynergyFinder tool revealed that independently, this analysis can predict the interaction patterns among anti-cancer bioactives on a cancer cell line when used in combinations, facilitating identification of synergistic, additive and antagonistic dose regions to effectively decide combination strategies. By exploring the previously published experimental datasets, this study overviews the graphical and numerical computational interpretations and highlights the value of data-driven approaches. The discussion findings focus that RSM and SynergyFinder serve distinct and vital roles in optimizing single formulations and multi-component systems, respectively. Overall, this work supports the adoption of AI-enabled methodologies as resourceful, and trustworthy tools which can be utilized for other pharmaceutical applications too as a promising tool to advance the development of neurotherapeutics and anticancer nutraceuticals.

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ABBREVIATIONS

AI: Artificial Intelligence; **BBD:** Box-Behnken Design; **BBB:** Blood-Brain Barrier; **CCD:** Central Composite Design; **CI:** Combination Index; **CNS:** Central Nervous System; **CQAs:** Critical Quality Attributes; **DoE:** Design of Experiments; **EE:** Entrapment Efficiency; **HPV:** Human Papillomavirus; **HSA:** Highest Single Agent; **NLCs:** Nanostructured Lipid Carriers; **OFAT:** One-factor-at-a-time; **PBCA:** Polybutyl Cyanoacrylate; **PDI:** Polydispersity Index; **PLGA:** Poly(lactic-co-glycolic acid); **ROS:** Reactive Oxygen Species; **RSM:** Response Surface Methodology; **SLNs:** Solid Lipid Nanoparticles; **ZIP:** Zero Interaction Potency; **ZP:** Zeta Potential

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Kalpana Nagpal: Conceptualization, methodology, data curation, formal analysis, visualization, investigation, resources, writing - review, and editing.

SUMMARY

This manuscript reviews the integration of artificial intelligence and computational modeling in pharmaceutical development. It specifically evaluates Response Surface Methodology (RSM) for statistically optimizing brain-targeted nanoparticle formulations and the SynergyFinder platform for determining the efficacy of anticancer bioactive combinations. The integration of these tools reduces experimental burdens, identifies synergistic dosage ratios, and enables the rational, data-driven design of targeted and safe pharmaceutical therapies.

PATENT DISCLOSURE

A provisional patent application titled "Synergistic Ellagic Acid-Quercetin Chewable Gummy Composition and Method for Preparation Thereof" has been filed by Amity University. The intellectual property covers the composition, the ratio-controlled incorporation of ellagic acid and quercetin, the extraction and purification workflow, and the controlled gummy formulation method. The patent claims the observed synergistic biological activity demonstrated through *in vitro* evaluations.

DECLARATION OF ARTIFICIAL INTELLIGENCE (AI) ASSISTANCE

I hereby declare that Artificial Intelligence (AI) tools, specifically *OpenAI's ChatGPT (GPT-5)*, were utilized solely for language refinement, formatting, data visualization guidance, and improvement of presentation clarity. No AI tool was used to generate experimental data, manipulate results, or alter scientific interpretations. All experimental work, data analysis, and conclusions presented in this document are the outcome of my own research and critical interpretation.

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