

A Three-Axis Mathematical Framework for Testing Proposed Complementary Actions of *Nigella sativa* and *Punica granatum* Peel Extract against *Helicobacter pylori*-associated Gastric Injury

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ABSTRACT

Chronic gastritis is a common condition associated with *Helicobacter pylori* (*H. pylori*) infection, which is also linked to peptic ulcer disease and gastric cancer. Natural products are being investigated as potential complements to current therapy, particularly because antimicrobial resistance, treatment failure, and tolerability remain important concerns. Constituents of *Nigella sativa* and *Punica granatum* have individually been reported to affect *H. pylori*, urease activity, inflammation, or oxidative stress, but the potential effects of combining the two plants have received little attention. This article proposes a three-axis mathematical framework for examining possible complementarity across bacterial survival and persistence, virulence and host interaction, and host and mucosal injury. Single-agent effects are represented by Hill concentration-response functions, while a Bliss-based interaction parameter is proposed for each endpoint to distinguish effects that are greater than, consistent with, or lower than the reference expectation for independent action. Differential equations link bacterial dynamics with host inflammation, oxidative stress, barrier integrity, and epithelial damage. A stability condition is also proposed for the inflammation-oxidative-stress feedback loop. Each model parameter is linked to a proposed assay, and decision rules are specified before data collection. The virulence axis remains exploratory because much of the current evidence is computational. This is a conceptual framework, not evidence of proven synergy, and it does not claim that either plant prevents gastric cancer.

Keywords: *Helicobacter pylori*; *Nigella sativa*; *Punica granatum*; Pomegranate Peel; Thymoquinone; Urease; Caga; Vaca; Bacterial Persistence; Mucosal Injury; Inflammation; Oxidative Stress; Autophagy; Molecular Docking; Network Pharmacology; Synergy Testing; Mathematical Model

INTRODUCTION

Helicobacter pylori is a helical, Gram-negative bacterium that colonizes the stomach. Infection is associated with chronic gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma [1, 2]. When infection persists, the gastric mucosa may remain inflamed, contributing to progressive tissue injury [1].

Standard treatment generally combines antibiotics with acid-suppressing drugs. However, treatment does not always eradicate the infection. Antimicrobial resistance, poor adherence, adverse effects, and regional differences in resistance patterns can reduce treatment success [2]. These challenges have encouraged interest in natural products as potential sources of anti-*H. pylori* compounds. A multi-component approach is biologically plausible because bacterial persistence involves several processes, including urease-mediated acid tolerance, epithelial adhesion through adhesins such as BabA and SabA, virulence-factor activity, and interactions with host tissue [1, 3, 4].

Many plants contain bioactive compounds that can influence infectious agents and may have anti-*H. pylori* or gastroprotective effects. *Nigella sativa* seeds contain thymoquinone (TQ), dihydrothymoquinone (DHTQ), thymol, and other constituents. Pomegranate peel is rich in hydrolysable tannins, ellagic acid, flavonoids, anthocyanins, and related polyphenols [5]. These

constituents have been investigated for effects on bacterial growth, urease activity, inflammatory signalling, oxidative stress, and mucosal injury [5, 6].

The studies cited here have generally examined one extract or compound in relation to a particular mechanism. Pomegranate peel preparations have been investigated for growth and urease inhibition, including in one study in combination with metronidazole [5, 7]. A delphinidin-rich pomegranate exocarp extract has been examined for effects on autophagy and cytokines, *N. sativa* has been studied in patients with *H. pylori* infection, and TQ and thymol have been evaluated computationally against CagA and VacA [6, 8, 9, 10]. The cited studies do not establish the effects of combining these two plants across bacterial persistence, virulence, and host injury.

This article brings together published evidence on the two plants to develop three mechanistic axes and identify the evidence available for each. It expresses the resulting hypotheses in a mathematical model in which interaction is a measurable quantity to be estimated experimentally, rather than a descriptive claim. Each parameter is linked to a proposed assay, and decision rules are specified before data collection so that combinations can be assessed against criteria defined in advance. The framework is a plan for future experiments and reports no new data. Its main aim is to provide a way of determining whether a given combination is additive, synergistic, indifferent, or antagonistic.

Rationale for a Three-Axis Strategy

The model consists of three interacting but conceptually distinct axes. This separation helps prevent reported activities from being interpreted as evidence for one unified mechanism and allows the proposed complementarity to be tested using endpoint-specific measurements. The rationale is that *H. pylori*-associated injury involves bacterial persistence, virulence-related interactions with host tissue, and host inflammatory and mucosal responses. These processes are connected, but they are not interchangeable; a change in one endpoint does not establish a change in the others.

Axis 1: Bacterial survival and persistence. This axis covers urease activity, bacterial growth, membrane integrity, and survival in biofilms.

Axis 2: Virulence and host interaction (exploratory). This axis addresses possible effects on virulence-associated processes, adhesion, colonization, and host-cell signalling.

Axis 3: Host and mucosal injury. This axis covers inflammatory signalling, oxidative stress, mucosal-barrier function, gastric acid secretion, and autophagy-related responses.

Evidence is considered at the level at which it was generated. Both plants have in vitro and extract-level evidence relevant to Axis 1. In vitro and animal studies of individual preparations inform Axis 3. For Axis 2, the evidence for *N. sativa* is mainly computational, and direct evidence for *P. granatum* is limited; this axis is therefore treated as exploratory.

The central hypothesis is that constituents of *N. sativa* and *P. granatum* may contribute different or complementary effects across these axes. This possibility should not be equated with pharmacological synergy. Synergy can be established only when experiments show that the combined effect exceeds the expectation defined by an appropriate reference model for the individual agents [11].

The axes are connected and their effects may be difficult to separate. For example, inflammatory markers may decrease because treatment reduces the bacterial load, rather than because the treatment directly modulates host inflammation. The experimental plan in Section 9 therefore proposes controls that hold bacterial load constant or use bacterial components to help distinguish host-directed effects from effects mediated by bacterial suppression.

The Role of *Nigella sativa*

Botanical profile and phytochemical composition

Black seed, also known as black cumin (*Nigella sativa*), belongs to the family Ranunculaceae. Its seeds contain volatile and fixed oils, alkaloids, flavonoids, saponins, and phenolic compounds. Thymoquinone is a major constituent of the volatile oil, while other reported constituents include dihydrothymoquinone (DHTQ), thymohydroquinone, thymol, carvacrol, p-cymene, α -pinene, and β -myrcene [12].

Axis 1: Bacterial survival and persistence

Thymoquinone (TQ) and dihydrothymoquinone (DHTQ) are candidate compounds for investigating effects on bacterial survival mechanisms [13]. However, their direct inhibitory activity against *H. pylori* urease has not been shown and requires confirmation using an appropriate enzyme assay. *N. sativa* extracts have also shown anti-urease activity in vitro, in a jack bean urease assay [14]. TQ has additionally been associated with antibacterial and antibiofilm activity in other bacterial species [15]. These findings support including *N. sativa* in the bacterial-survival axis, while the individual mechanisms still need to be assessed separately.

Urease inhibition and antibiofilm activity are distinct mechanisms and should not be treated as interchangeable. Future experiments should determine whether specific *N. sativa* constituents affect urease activity, biofilm formation, or both.

Clinical evidence for *N. sativa* in *H. pylori* eradication remains limited. Salem et al. compared *N. sativa* with standard triple therapy in patients with dyspepsia, while a later controlled trial examined *N. sativa* powder as an adjunctive treatment [8, 9]. These studies provide a rationale for further investigation but do not establish the efficacy of the proposed combination with pomegranate peel.

Axis 2: Virulence and host interaction

Docking studies have predicted interactions between TQ or thymol and the *H. pylori* virulence-associated proteins CagA and VacA [10]. These predictions identify candidates for further study, but docking scores do not demonstrate that either protein is inhibited in a living system. The compounds should therefore be evaluated using appropriate virulence assays and host-cell signalling studies.

Future work should examine bacterial adhesion, *cagA* and *vacA* expression, CagA translocation, and relevant host-cell signalling rather than relying on docking alone. Reports of anti-adhesive effects from other culinary and medicinal plants support including adhesion assays in the proposed validation plan [16].

Axis 3: Host and mucosal injury

TQ has been shown to inhibit gastric acid secretion, decrease neutrophil infiltration, and enhance mucin secretion and nitric oxide production [17]. *Nigella sativa* and its constituent thymoquinone have been discussed in the literature for their potential anti-inflammatory properties, including possible modulation of inflammatory mediators. The extent to which these effects influence TNF- α , IL-1 β , and IL-8 during *H. pylori*-associated gastric injury requires further investigation [13]. These reports indicate that there may be a protective effect on the host.

Host-directed effects should be distinguished from direct bacterial killing. Inflammatory signalling may change even when *H. pylori* is not eliminated, so host protection would complement rather than replace bacterial suppression in the model. The proposed antioxidant effects also need to be considered alongside the antibacterial activity attributed to reactive oxygen species (ROS) generation by TQ. These effects may differ by cell type and concentration—bacteria versus host cells—and should be tested rather than assumed.

Proposed Role of *Punica granatum*

Pomegranate peel phytochemical composition

Punica granatum L. has a high polyphenol content, particularly in its peel. Compounds reported in pomegranate peel include punicalagins A and B, punicalin, ellagic acid, gallic acid, catechins, other flavonoids, and anthocyanins such as delphinidin-3-O-glucoside, cyanidin-3-glucoside, and pelargonidin-3-glucoside [5].

Axis 1: Bacterial survival and persistence

H. pylori in vitro inhibition by a pomegranate peel ethanol extract (PPEtE) has been reported, and the proposed mechanisms of action include membrane disruption and metal ion chelation [5]. The same extract inhibited urease activity [5]. In addition, partially purified extracts from pomegranate peel have been found to inhibit the growth of *H. pylori* [7]. This will facilitate testing of pomegranate constituents in combination with *N. sativa* constituents on multiple targets of bacterial survival.

The concentrations in the Mayyas study are of interest. The MIC of the extract was 0.156 mg/mL and its urease inhibition was 6 mg/mL (IC₅₀) [5]. If the same is true for other preparations, then it appears that urease inhibition does not account for the majority of the growth effect at the concentrations studied because they are approximately 40 times lower than those required to inhibit the enzyme. The difference between the two assays is noted only to suggest running both assays on the same sample. It does not indicate that urease is the primary target of pomegranate.

Evidence and conclusions should be attributed to the preparation or compound actually tested. Results obtained with a whole-peel extract should not automatically be attributed to individual compounds; those compounds require their own experiments.

Axis 2: Virulence and host interaction

The pomegranate peel preparations can influence the bacteria-host interaction indirectly through their ability to reduce the viability of the bacteria, and also through altering the host response. As such, the evidence presented in the sources cited here is more robust for bacterial inhibition, as well as host inflammatory and autophagy responses, than for direct inhibition of a specific virulence protein of *H. pylori*. For this axis, therefore, the evidence for *P. granatum* is indirect, and whether it has a direct effect remains an open question.

In view of this, other proteins of *H. pylori* that have been shown to play roles in adhesion and persistence, namely the adhesins BabA and SabA and the efflux-associated protein HefA are also included in the proposed computational plan as candidate targets.

Axis 3: Host and mucosal injury

It has been reported that Pomegranate exocarp extract can induce autophagy and reduce the expression of TNF- α and COX-2. Pomegranate peel ethanol extract has been found to reduce *H. pylori*-induced gastritis, which is believed to be due to its ROS scavenging, mucosal protection and inhibition of inflammatory cascades. One exocarp preparation containing delphinidin-3-O-

glucoside was investigated in particular for its anti-*H. pylori* activity, increased autophagy and decreased levels of TNF- α and COX-2. Pomegranate has also been shown to have gastroprotective and ulcer therapeutic properties on stomach ulcer caused by *H. pylori* [18].

The Three-Axis Complementary Mechanistic Model

Axis 1: Bacterial Survival and Persistence

TQ and DHTQ from *N. sativa* have been proposed to affect urease and biofilm formation. Pomegranate-peel polyphenols and tannins have been associated with growth inhibition, membrane effects, and urease inhibition. Because both plants may affect urease, this overlap limits the argument for complementarity at that target alone. Two inhibitors of the same enzyme may produce an additive effect, or compete if they bind to the same site. Complementary effects on urease would need to be demonstrated—for example, if the compounds act at different sites or different stages of enzyme function, such as catalytic activity or nickel delivery during enzyme maturation [19]. Different predicted binding modes, on their own, do not demonstrate synergy.

The enzyme-kinetics experiments described in Section 9.2, together with studies of biofilm formation, membrane effects, and bacterial growth, may therefore provide more informative tests of complementarity on this axis.

The proposed pathway is that one or both extracts may affect urease activity, bacterial growth, membrane integrity, or biofilm formation, thereby reducing bacterial survival or persistence. Which of these effects occurs, and whether the combination improves on either agent alone, remains to be tested.

Axis 2: Virulence and Host Interaction (exploratory)

TQ and thymol are candidates for experimental evaluation of virulence-associated processes because computational studies have predicted interactions with *CagA* and *VacA*. Pomegranate constituents might contribute indirectly by reducing bacterial viability or altering host responses. One hypothesis is that one group of compounds could affect virulence-related activity while the other affects bacterial persistence or host injury. This possibility should be tested using adhesion assays, virulence-gene expression measurements, *CagA/VacA*-related assays, and appropriate host-cell models. Until such data are available, this axis represents proposed measurements rather than established findings.

Conceptual Pathway: reduced pathogen–host contact, whether through altered virulence-associated activity or a lower bacterial burden, may reduce sustained stimulation of the gastric epithelium.

Axis 3: Host and Mucosal Injury

The third axis concerns the host response to infection. In rodent gastric-injury models, *N. sativa* has been reported to increase mucin, modulate gastric acid secretion, and reduce inflammatory mediators and neutrophil infiltration [19]. Pomegranate preparations have been associated with antioxidant activity, lower TNF- α and COX-2 expression, autophagy-related responses, and mucosal protection. These reported effects suggest possible convergence on oxidative stress and inflammatory injury, but the effects of the combination remain untested.

Conceptual pathway: the combination may reduce inflammatory signalling and oxidative stress and support mucosal defence and autophagy-related responses. Whether these effects are additive or synergistic, and whether they reduce epithelial injury, must be established experimentally.

Integration of the Three Axes

The axes are biologically connected and should not be represented as a single linear pathway. Reduced bacterial persistence may lessen ongoing inflammatory stimulation; reduced virulence-associated activity may limit epithelial damage; and improved host defences may help reduce tissue injury. Together, these processes could influence the course of chronic *H. pylori*-associated gastritis. Their possible relationship to later carcinogenesis is shown as downstream biological context, not as evidence that the proposed combination prevents cancer.

Any relationship with cancer would be indirect and remains hypothetical. The proposed model does not describe a direct effect on cancer cells, and current evidence presented here does not establish that *N. sativa* or *P. granatum* prevents or treats gastric cancer.

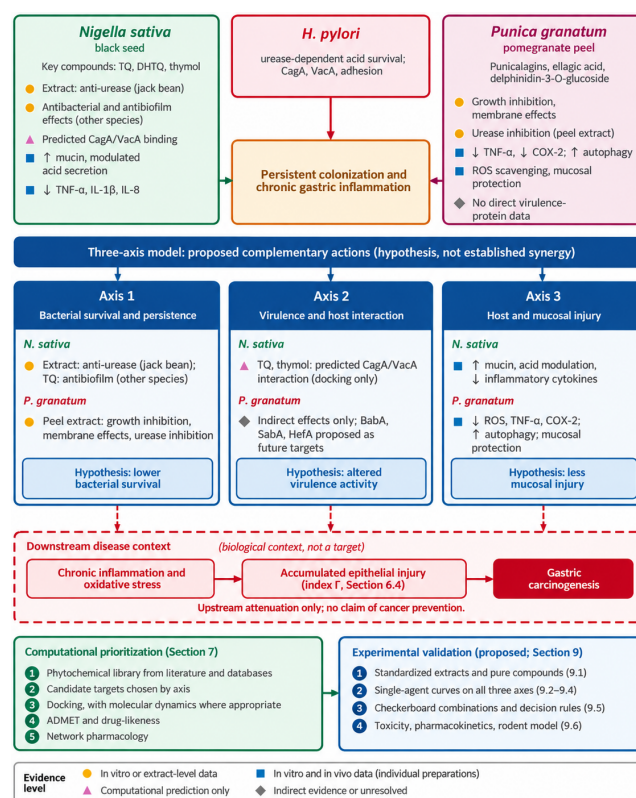


Figure 1: Proposed three-axis model linking *Nigella sativa* and *Punica granatum* bioactives with *H. pylori* persistence, virulence and host interaction, and gastric mucosal injury. The downstream carcinogenesis pathway is shown as biological context and is not evidence of direct cancer prevention

Dashed lines indicate the possible downstream relationship with disease progression. Epithelial injury corresponds to the index Γ in Section 6.4. Marker shapes and colours indicate the evidence level described in the key. Axis 2 is based mainly on computational predictions and is therefore exploratory.

Mathematical Formulation of the Model

This section expresses the three-axis framework as a set of equations intended to make complementarity and interaction measurable in future experiments. The equations are proposed models, not observed biological facts. Each compound's effect on an endpoint is represented by a saturating concentration–response curve, and the combined response includes an endpoint-specific interaction parameter. No parameter values have been fitted because the study reports no new experimental data. Section 6.5 and Table 1 describe how the parameters could be measured and estimated. Figure 2 summarizes the relationships among the model variables.

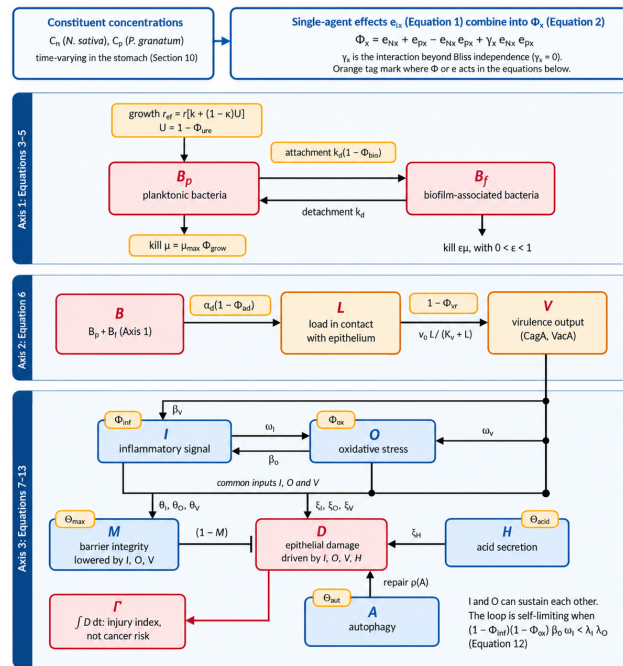


Figure 2: Structure of the model described in Section 6. Boxes represent state variables, and black arrows show the couplings in Equations 3–11. The horizontal bar collects I , O and V , which act on barrier integrity M (Equation 9) and on damage D (Equation 11); the flat-ended line shows M limiting damage. Orange tags indicate where the combined effect Φ (Equation 2) or a single-agent effect e (Equation 1) acts. Total bacterial load B also limits growth through the carrying capacity K . The index Γ represents accumulated injury, not cancer risk

Concentration–Response and Combination

Let C_N and C_P denote the concentrations of a constituent from *Nigella sativa* (e.g., thymoquinone, TQ) and a constituent from *Punica granatum* (e.g., punicalagin), respectively. For compound and endpoint, the normalized single-agent effect, ranging from 0 to 1, is represented by the following Hill-type concentration–response equation:

$$e_{i,x} = \frac{C_i^h}{EC50_{i,x}^h + C_i^h} \quad (1)$$

Here, $EC50_{i,x}$ is the concentration of compound that produces half of its maximum effect on endpoint, and is the Hill coefficient, which describes the steepness of the concentration–response relationship. The Hill coefficient may differ between compounds and endpoints, and the subscripts i and x are omitted from h in Equation 1 for brevity. The endpoints considered in this framework are urease activity (ure), bacterial growth or survival (grow), biofilm formation (bio), bacterial adhesion (adh), virulence output (vir), inflammation (inf), oxidative stress (ox), mucin-related protection (muc), gastric acid secretion (acid), and autophagy (aut). The equation provides a proposed mathematical representation of the single-agent effects; its parameters must be determined experimentally.

Equation 1 is an illustrative starting point and does not assume that every compound produces complete inhibition or stimulation. If experiments indicate a partial maximum effect, the fitted model should include an endpoint-specific maximum-effect term. The direction and scale of each response must be defined before combining effects. The combined effect of both compounds on endpoint is then represented as follows:

$$\Phi_x = e_{N,x} + e_{P,x} - e_{N,x} e_{P,x} + \gamma_x e_{N,x} e_{P,x} \quad (2)$$

When $\gamma_x=0$, the equation represents Bliss independence, in which the agents act independently under the chosen response scale [Bliss, 1939]. A positive γ_x indicates a response greater than the Bliss reference prediction, whereas a negative γ_x indicates a response below that prediction. Each endpoint has its own γ_x , allowing interaction to differ across endpoints. The exceptions are mucin-related protection and autophagy, where Equations 9 and 10 use additive terms (Section 6.4). Values of γ_x are specific to the endpoint and concentration range and describe deviations from the Bliss prediction; they do not, by themselves, establish general biological or clinical synergy.

Where the cited sources do not report an effect for a compound on an endpoint, the base case sets the corresponding term to zero ($e_{(P,bio)}$, $e_{(P,adh)}$, $e_{(P,vir)}$, $e_{(P,acid)}$, and $e_{(N,aut)}$). This represents a lack of cited evidence, not proof of no effect; each term would need to be measured.

The predicted combined response is intended to lie within the interval 0–1. However, Equation 2 does not automatically guarantee this range. If the model produces a value outside this interval, the prediction will be constrained to the nearest boundary. Because this constraint can affect the estimation of the interaction parameter, γ_x should be estimated using unsaturated observations, and the effect of the constraint on model predictions should be assessed during validation.

Bliss independence is used here as the default reference because the two extracts contain many constituents that probably act on different targets. It is less appropriate where both may act on the same target, as may be the case for urease (Section 5.1). For that endpoint, the combination will also be analysed against Loewe additivity, using isobolograms and the FIC index, and the interaction will be reported relative to both references. A combination will be called promising only if it exceeds the expectation under both models.

Axis 1: Bacterial survival and persistence

Let B_p be planktonic bacteria, B_f biofilm-associated bacteria, $B = B_p + B_f$, and U the urease activity relative to untreated bacteria. Then

$$U = 1 - \Phi_{ure}, \quad r_{eff} = r [\kappa + (1 - \kappa) U], \quad \mu = \mu_{max} \Phi_{grow} \quad (3)$$

$$dB_p/dt = r_{eff} B_p (1 - B/K) - k_a (1 - \Phi_{bio}) B_p + k_d B_f - \mu B_p \quad (4)$$

$$dB_f/dt = k_a (1 - \Phi_{bio}) B_p - k_d B_f - \epsilon \mu B_f \quad (5)$$

The parameter represents the bacterial growth rate when urease is fully active, while is the carrying capacity. The parameters K_a and K_d represent the biofilm attachment and detachment rates, respectively, and ϵ , where $0 < \epsilon < 1$ represents the reduced susceptibility of biofilm-associated cells to the bacterial-loss process. In this base model, biofilm-associated cells are assumed not to divide; therefore, Equation 5 contains no growth term. Although biofilm-associated cells may grow more slowly than planktonic cells, this process is omitted to keep the model parsimonious. The biofilm compartment consequently acts as a protected reservoir that gains cells through attachment and loses them through detachment and bacterial loss. If experimental data indicate that growth within biofilms is important, a term $r_f B_f$, where $r_f < r$, could be added to Equation 5.

Three limitations apply to Equations 3–5. First Φ_{grow} , enters the model only through μ , which acts as a bacterial-loss rate. Therefore, in this base formulation, Φ_{grow} represents an antibacterial effect modelled as bacterial loss rather than general bacteriostatic growth inhibition. Future experiments should distinguish changes in bacterial growth rate from bacterial killing, and the model should be extended if these processes differ. Second, the relationship between urease inhibition and bacterial growth applies only under the specified pH and urea conditions. The parameter represents the residual fraction of the growth rate when urease is fully inhibited under those conditions; it should not be interpreted as a universal constant. Third, the term involving Φ_{bio} represents reduced transfer of planktonic bacteria into the biofilm compartment. It does not describe the disruption or killing of an already established biofilm. These assumptions require evaluation during future experimental validation.

Axis 2: Virulence and host interaction

$$L = \alpha_0 (1 - \Phi_{adh}) B, \quad V = v_0 (1 - \Phi_{vir}) L / (K_L + L) \quad (6)$$

L is the bacterial load in contact with the epithelium, α_0 is the untreated contact fraction, V is the virulence-related output associated with CagA and VacA, v_0 is the maximum value of V , and K_L is the contact load at which V reaches half of v_0 . In the base case, pomegranate-associated effects on adhesion or virulence are not assumed in the absence of direct evidence; any such effects must be measured. Bacterial killing also reduces L , so the effects represented by $e_{N,adh}$ and $e_{N,vir}$ should be measured at concentrations that do not affect bacterial growth.

Axis 3: Host and mucosal injury

Let I be the pooled inflammatory signal (TNF- α , IL-1 β , IL-8, COX-2), O oxidative stress in host cells, M mucosal barrier integrity (0 to 1), A autophagy activity and H gastric acid secretion relative to untreated and D an epithelial damage index. Then

$$dI/dt = (\sigma_I + \beta_V V + \beta_O O)(1 - \Phi_{inf}) - \lambda_I I \quad (7)$$

$$dO/dt = (\sigma_O + \omega_I I + \omega_V V)(1 - \Phi_{ox}) - \lambda_O O \quad (8)$$

$$dM/dt = \kappa_M (1 + m_N e_{N,muc} + m_P e_{P,muc})(1 - M) - (\theta_I I + \theta_O O + \theta_V V) M \quad (9)$$

$$dA/dt = \sigma_A (1 + a_N e_{N,aut} + a_P e_{P,aut}) - \lambda_A A \quad (10)$$

$$dD/dt = (\xi_I I + \xi_O O + \xi_V V + \xi_H H)(1 - M) - \rho(A) D \quad (11)$$

In Equation 8, σ_O represents baseline oxidative-stress input ω_I and ω_V , represent the contributions of inflammation and virulence-related output to oxidative stress, and λ_O represents oxidative-stress resolution. The factor $(1 - \Phi_{ox})$ represents the proposed reduction in oxidative-stress input associated with antioxidant activity in host cells.

In Equation 9, κ_M represents the baseline rate of barrier restoration, while m_n and m_p represent the proposed contributions of *Nigella sativa* and *Punica granatum* to mucosal protection. The parameters θ_I , θ_O and θ_V and represent the contributions of

inflammation, oxidative stress, and λ_O virulence-related output to barrier impairment, respectively. The variable M is normalized between 0 and 1, with higher values representing greater barrier integrity.

In Equation 10, σ_A represents baseline autophagy input a_N , and a_P represent the proposed effects of the two plant-derived interventions on autophagy activity, and λ_A represents the rate of autophagy-signal decay. These terms describe a proposed relationship and do not establish that either intervention increases protective autophagic activity.

In Equation 11 ξ_i , ξ_o , ξ_v and ξ_H represent the contributions of inflammation, oxidative stress, virulence-related output, and gastric acid secretion to epithelial damage. The factor $(1-M)$ represents the increased susceptibility to damage associated with reduced barrier integrity. The function $\rho(A)=\rho_0+\rho_1 A/(K_A+A)$ describes a proposed increase in the damage-resolution rate with increasing autophagy activity. Here ρ_0 is the baseline repair rate, ρ_1 is the maximum additional repair-rate contribution, and K_A is the autophagy activity at which this additional contribution reaches half its maximum. The expression $H=H_0 (1-\eta e^{-(N_{acid})})$ represents the proposed effect of *Nigella sativa* on gastric acid secretion, where H_0 is the untreated acid-secretion level and η represents the magnitude of the modeled effect.

To distinguish bacterial and host-cell effects of thymoquinone (TQ), its proposed pro-oxidant effect on bacteria, if supported by the relevant evidence, should be evaluated separately from its proposed antioxidant effect in host cells. The corresponding effects should be measured in appropriate bacterial and host-cell systems rather than assumed to be identical. The parameters and relationships in Equations 8–11 are conceptual and require experimental validation.

Two simplifications apply to Equations 9 and 10. First, the effects of the two plants enter through additive terms ($m_N e_{N,muc} + m_P e_{P,muc}$ and $a_N e_{N,aut} + a_P e_{P,aut}$) rather than through the combined effect Φ of Equation 2. No interaction parameter γ is therefore defined for mucin-related protection or autophagy, and in the base case these two endpoints are treated as additive. An interaction could be added later by replacing each sum with Φ_{muc} or Φ_{aut} . Second, $e_{N,aut}$ is set to zero in Section 6.1 because the cited sources report no autophagy effect of *N. sativa*, so the a_N term is kept only for use if such an effect is measured. Host-cell viability, written Q in Section 8 and Table 1, is an experimental readout alongside CC50 and is not a state variable in Equations 7–11.

Two properties follow. First, inflammation and oxidative stress can sustain each other through a positive feedback loop. With V and Φ_{inf} and Φ_{ox} held constant, the linearized inflammation–oxidative-stress subsystem is locally asymptotically stable if

$$(1 - \Phi_{inf})(1 - \Phi_{ox})\beta_O\omega_I < \lambda_I\lambda_O,$$

assuming positive resolution rates and non-negative feedback coefficients. This condition indicates that the combined strength of the feedback between inflammation and oxidative stress must remain below the stabilizing effect of their respective resolution rates. It applies to this subsystem of the proposed model and does not demonstrate that the plant combination has experimentally stabilized these processes.

$$(1 - \Phi_{inf})(1 - \Phi_{ox})\beta_O\omega_I < \lambda_I\lambda_O \quad (12)$$

A treatment that increases Φ_{inf} and Φ_{ox} could move the system below this threshold, providing one possible mathematical representation of reduced chronic injury. Accumulated injury over a treatment interval T is represented by Equation 13. Γ is an injury index used as downstream biological context in Figure 1; it is not a measure of cancer risk, and the model makes no claim about carcinogenesis.

$$\Gamma(T) = \int_0^T D(t) dt \quad (13)$$

Classifying interactions and measuring parameters

For each endpoint, the interaction parameter is estimated from a concentration matrix using the observed combined effect e_{obs} :

$$\gamma_x = (e_{obs} - e_{N,x} - e_{P,x} + e_{N,x} e_{P,x}) / (e_{N,x} e_{P,x}) \quad (14)$$

The observed combined response, e_{obs} , is used to estimate the interaction parameter, γ_x , in Equation 2. Before estimating this parameter γ_x , each endpoint should be normalized using a predefined response scale and direction convention to ensure consistent interpretation across different biological endpoints. The estimated value of γ_x represents the deviation from the Bliss-independent expectation under the specified model assumptions; it does not, by itself, establish statistically reliable or clinically meaningful synergy.

The estimate in Equation 14 is undefined when either single-agent effect is zero and becomes unstable when either effect approaches zero. Furthermore, clipping the combined response in Equation 2 to the interval 0–1 creates an additional limitation because cannot generally be recovered reliably from observations at which the response has saturated. Therefore, γ_x should be estimated using concentrations at which both single-agent effects are measurable and the combined response remains unsaturated. Fitting a concentration–response surface and reporting uncertainty intervals are preferable to interpreting isolated point estimates. These procedures are proposed for future experimental validation and do not represent results obtained in the present conceptual study.

For validation, single-agent concentration–response curves should first be fitted and checked for adequate model fit. Combination responses should then be measured across a concentration matrix and compared with the Bliss-independent expectation using the same predefined endpoint scale. The interaction parameter should be estimated with uncertainty intervals and examined across independent experiments and extract batches. Where feasible, model predictions should also be compared with an independent set of observations. If the model fails to describe the single-agent data, its assumptions or functional form should be revised before interpreting combination results.

For bacterial growth, the fractional inhibitory concentration (FIC) index can also be reported: $FIC = (MIC_{N,comb}/MIC_{N,alone}) + (MIC_{P,comb}/MIC_{P,alone})$, where each combination MIC is divided by the MIC of the corresponding agent alone. Under commonly used criteria, $FIC \leq 0.5$ indicates synergy, values >0.5 to 4 indicate no clear interaction, and values >4 indicate antagonism [20]. The intermediate range does not distinguish additivity from indifference reliably; the Bliss-based γ_x therefore provides a complementary measure under its stated assumptions. If sufficient data are available, γ_x could be modelled as a function of C_N and C_P . Table 1 links each parameter group to a proposed assay, and the hypotheses in Section 8 are expressed in terms of these parameters.

Parameters	Meaning	Measured by
EC50 and h for each compound and endpoint (Equation 1)	Potency and steepness of each single-agent effect	Concentration–response curves for each endpoint in Sections 9.2 to 9.4
γ for each endpoint (Equations 2 and 14)	Interaction between the two plants on that endpoint	Concentration matrix (checkerboard) data; FIC index; Bliss excess
r, K, κ, μ_{max}	Growth, capacity, growth kept in acid without urease, maximum kill rate	Growth curves, time-kill curves, growth at low pH with and without urea
k_a, k_d, ε	Attachment to and detachment from biofilm; reduced susceptibility of biofilm	Crystal-violet staining; viable counts in biofilm and planktonic fractions
α_0, v_0, KL	Contact fraction, maximum virulence output, half-saturation load	Adhesion assays, <i>cagA/vacA</i> RT-qPCR, CagA translocation, vacuolation
$\sigma, \beta, \omega, \lambda$ for I and O	Production, coupling and decay of inflammation and oxidative stress	ELISA time courses, ROS probes in infected host cells
$\kappa M, m, \theta$	Barrier renewal, mucin effect, damage to barrier	Mucin-associated expression, barrier resistance
$\sigma A, a, \rho, \lambda A, \rho_0, \rho_1, KA$	Autophagy induction and repair	Beclin-1, ATG5, ATG12 levels, injury and repair readouts
ξ, η, H_0	Damage coupling and acid effect	Cell injury markers; animal histology
CC50, Q	Host-cell toxicity and viability	Host-cell viability assays

Table 1: Model Parameters and How Each Would Be Measured

Proposed compound-target-pathway approach

The computational component should help assess the internal consistency of the three-axis model and prioritize candidates for testing; it cannot by itself validate biological effects. First, compile a library of phytochemicals reported for *N. sativa* and *P. granatum* using published literature and relevant chemical or biological databases. Second, select candidate *H. pylori* targets based on the three axes. Third, use molecular docking to explore plausible compound–target interactions and, where appropriate, molecular dynamics to examine the stability of predicted interactions. Fourth, use absorption, distribution, metabolism, excretion, and toxicity (ADMET) and drug-likeness analyses to prioritize candidates for further study. Finally, use network pharmacology to examine whether the predictions converge on pathways related to bacterial survival and virulence, inflammation, oxidative stress, and autophagy [21, 22].

Where possible, docking should be checked before interpretation—for example, by re-docking co-crystallized ligands and reporting the deviation from the crystal pose. Urease docking should explicitly account for its nickel centre. High docking scores require careful scrutiny because polyphenols and quinones can produce nonspecific or misleading hits; a score is not proof of specific binding. Computational outputs may help prioritize compounds and suggest concentration ranges for subsequent experiments, but they are not measured parameters and cannot replace experimental EC50 estimates. The evidence for each axis is summarized in Table 2.

Axis	<i>N. sativa</i>	<i>P. granatum</i>	Current evidence	Key validation question
1. Bacterial survival and persistence	TQ/DHTQ: urease inhibition; TQ: antibiofilm activity	Peel extract/polyphenols: growth inhibition,	In vitro/extract evidence; some docking. Urease is a shared target	Do the compounds act at different urease sites? Does the combination

		membrane effects, urease inhibition		inhibit growth, urease and biofilm more strongly than individual agents?
2. Virulence and host interaction (exploratory)	TQ/thymol: predicted CagA/VacA interactions	Possible indirect effects through bacterial suppression and host response; BabA, SabA and HefA proposed as targets	Mainly computational for CagA/VacA; no direct data for <i>P. granatum</i>	Does treatment alter adhesion, <i>cagA/vacA</i> expression or virulence-associated phenotypes at sub-MIC concentrations?
3. Host and mucosal injury	Increased mucin; acid modulation; reduced inflammatory signaling	ROS reduction; ↓TNF- α /COX-2; autophagy-related effects; mucosal protection	In vitro/in vivo evidence for individual preparations	Does the combination reduce ROS, inflammation and epithelial injury at equal bacterial load and without cytotoxicity?

Table 2: Three-axis Evidence and Proposed Mechanisms

Research hypotheses generated by the model

H1: Selected constituents of *N. sativa* and *P. granatum* inhibit *H. pylori* growth more effectively in combination than individually ($\gamma_{\text{grow}} > 0$ under the response convention defined in Equation 2, with $\text{FIC} \leq 0.5$). This is a hypothesis to be tested, not an established result.

H2: Constituents from the two plants may affect different sites or mechanisms within the urease and bacterial-survival system. This hypothesis should be evaluated by measuring enzyme-inhibition mechanisms in the presence of each compound and their combination and estimating γ_{ure} ; the presence of both agents alone does not establish complementary action.

H3: Selected constituents, alone and in combination, may reduce biofilm formation or bacterial persistence. Because the cited evidence does not establish anti-biofilm activity for pomegranate in the context considered here, e_{Pbio} must first be measured before γ_{bio} can be interpreted.

H4 (exploratory): *N. sativa* constituents predicted to interact with CagA or VacA may alter virulence-associated phenotypes at concentrations below the MIC, without affecting bacterial growth. This would be reflected by measurable values of $e_{\text{N,adh}}$ or $e_{\text{N,vir}}$.

H5: The combination may suppress *H. pylori*-associated inflammatory and oxidative responses more effectively than either treatment alone ($\gamma_{\text{inf}} > 0$ and $\gamma_{\text{ox}} > 0$), when bacterial load is held constant.

H6: The combination may reduce gastric epithelial injury relative to *H. pylori* infection alone, as indicated by lower D and Γ values and host-cell viability (Q) at or above a threshold established before the experiments.

H7: The interaction may be additive, synergistic, indifferent, or antagonistic depending on the compounds, endpoint, and concentration. Synergy should not be assumed; it must be demonstrated using experimental data and a predefined reference model.

Experimental validation pathway

To evaluate the framework, the computational predictions must be translated into measurable endpoints. Each compound should first be tested separately to establish MIC values and concentration–response relationships. Selected combinations can then be assessed using checkerboard assays and appropriate complementary metrics to distinguish additive, synergistic, indifferent, and antagonistic effects [11].

Materials and standardization

Plant material should be authenticated by a qualified botanist, a recognized herbarium, or both, and a voucher specimen should be deposited. A standardized extraction protocol should be used, and preparations should be characterized for TQ in *N. sativa* and punicalagin and ellagic acid in pomegranate peel. Doses should be reported both by marker-compound content and by dry extract weight. To distinguish extract-level effects from compound-specific effects, purified TQ, thymol, punicalagin, and ellagic acid should be tested alongside the extracts. Extraction should be repeated using at least two independent batches. Reference strains, such as NCTC 11916 used by Mayyas, and clinical isolates with different *cagA* and *vacA* status and different metronidazole and clarithromycin resistance profiles should be included. An isogenic *cagA*-deletion mutant can serve as a negative control for relevant Axis 2 assays.

Axis 1 Assays

Bacterial growth should be assessed using the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill curves under suitable microaerophilic conditions. Urease assays should include controls such as a thiol protector and bovine serum albumin, because tannins can bind proteins nonspecifically and interfere with apparent inhibition. The inhibition mechanism for each compound and combination can be examined using enzyme-kinetic analyses; compounds with different modes of inhibition may be candidates for complementary action, but this must be tested. Urease inhibition and growth inhibition should be compared at the same concentrations of each preparation. Biofilm effects should be assessed using crystal-violet staining and viable counts from both biofilm-associated and planktonic fractions.

Axis 2 Assays

Adhesion to gastric epithelial cells (for example, AGS cells) should be measured using appropriate viable-count or fluorescence-based assays. *cagA* expression can be assessed by RT-qPCR, *CagA* translocation and phosphorylation by immunoblotting, and *VacA* activity by a vacuolation assay. These tests should use concentrations below the MIC, with growth curves confirming that bacterial growth is not affected; this helps distinguish changes in virulence-related readouts from reductions caused simply by fewer bacteria. Isogenic mutant controls should be used for readouts that depend on the relevant target protein.

Axis 3 Assays

Gastric epithelial cells should be exposed to the individual compounds and the proposed combinations, and the selected endpoints should then be measured. These may include IL-8, TNF- α , and IL-1 β by ELISA; COX-2 expression; intracellular ROS; barrier function, assessed by transepithelial electrical resistance in polarized monolayers; autophagy markers such as Beclin-1, ATG5, and ATG12; and cytotoxicity (CC50). Two controls can help separate host-directed effects from Axis 1 effects. First, cells can be challenged with a predetermined, equal number of live bacteria across treatment groups. Second, heat-killed bacteria or bacterial lysate can be used to induce host responses without bacterial survival being a variable. ROS should be measured separately in bacteria and host cells, given the possibility that TQ has different effects in the two settings.

Combination Analysis

Single-agent concentration–response curves should first be fitted using Equation 1, based on at least three independent experiments with technical replicates. Combinations should then be tested using a concentration matrix of at least six by six concentrations and analyzed using the FIC index [Odds, 2003] and Bliss excess with bootstrap confidence intervals. For the urease endpoint, the interaction will also be analysed against Loewe additivity, and results will be reported relative to both reference models. Acceptance criteria and other thresholds should be defined in the protocol before data collection. A combination could be prioritized for further study if it meets all of the following criteria: (1) $FIC \leq 0.5$ in two strains and a Bliss-excess 95% confidence interval above zero; (2) a selectivity index ($CC50/EC$, where EC is the effective concentration for the endpoint being tested) of at least 10 for both agents at the active concentration; (3) reproducibility with an independent extract batch; and (4) no evidence of assay interference based on the controls described above. Results that do not meet these criteria should still be reported and classified, where supported by the data, as additive, indifferent, or antagonistic.

Animal Studies

If in vitro findings justify further study, pharmacokinetic and toxicity assessments could precede testing in a rodent *H. pylori* infection model. Animal studies should follow ARRIVE 2.0 guidance, with a power-based sample size, randomization, and blinded histological assessment [24]. The design should include a standard-therapy group and groups receiving each agent individually. Outcomes could include bacterial load, gastritis, mucosal injury, and, in longer studies, premalignant changes. Clinical investigation should be considered only after sufficient preclinical evidence is available.

Gastric Conditions and Formulation

The model treats compound concentration as a controllable input, but concentration at the site of infection in the stomach cannot simply be assumed. *H. pylori* is found mainly in the mucus layer and on the epithelium, where the pH is closer to neutral than in the highly acidic gastric lumen [25]. A compound must survive passage through the lumen, penetrate the mucus, and remain active there for a sufficient period. Three practical issues therefore require attention.

Stability: TQ and some polyphenols may change under acidic conditions or during digestion. Simulated-digestion studies have been used to assess other natural products with anti-*H. pylori* activity; similar testing should be conducted for both plants before combination experiments [26].

Delivery: TQ has low water solubility, and tannins can bind proteins such as mucin. Whether the two plant fractions reduce one another's free concentration should be tested directly, for example by measuring the binding of TQ to the tannin fraction [27].

Residence time: an unformulated dose may leave the stomach within hours. Mucoadhesive or lipid-based formulations could be investigated, but the release profile of each formulation and an appropriate formulation control should be established.

These effects can be represented in the model through time-varying concentrations $CN(t)$ and $CP(t)$, which should be measured rather than assumed. Until these measurements are available, in vitro potency should not be interpreted as a prediction of potency in the stomach.

Pharmaceutical and Research Significance

The proposed model may help generate testable predictions and prioritize experiments on natural compounds relevant to *H. pylori*-associated injury. It does not assume that a complex plant extract is synergistic; instead, it identifies compounds, targets, pathways, and outcomes that can be investigated individually and in combination. Computational screening may help narrow the number of candidates before laboratory testing, while combination assays can determine whether the proposed complementarity translates into measurable pharmacological interaction [28].

The framework may also inform antimicrobial-resistance research by focusing on structurally diverse compounds and potential multi-target mechanisms. Plant-derived products should remain investigational until their composition, efficacy, safety, pharmacokinetics, and reproducibility have been established.

LIMITATIONS

Molecular docking provides theoretical predictions of ligand–protein interactions but cannot reproduce the complete biological environment of *H. pylori* infection.

A docking interaction with *CagA*, *VacA*, urease, or another protein does not demonstrate functional inhibition.

The effects of whole extracts are not necessarily the same for specific phytochemicals.

Apparent urease inhibition by pomegranate extracts may partly reflect nonspecific protein binding by polyphenols and tannins. Appropriate protein-binding and assay-interference controls are therefore necessary.

Thymoquinone has low water solubility and is poorly bioavailable, which could limit its activity in the stomach.

Bioactive compounds are not necessarily synergistic when combined; direct combination experiments are required.

Computational predictions (ADMET and network-pharmacology) may not agree with experimental pharmacology and toxicity.

The proposed host-protective pathways may be connected, but causal relationships among them have not been established for the combination.

The mathematical model has no fitted parameters. Its Hill curves, Bliss-based combination equation, logistic growth assumptions, use of one representative compound from each plant, and one interaction parameter per endpoint are simplifications. These assumptions should first be assessed against single-agent data before the model is used to interpret combination effects.

The axes are interconnected, so an apparent effect on one axis may arise from a change in another. The controls proposed in Sections 9.3 and 9.4 may reduce, but cannot eliminate, this limitation.

The reference model affects what counts as synergy. Bliss independence is a poor fit where both extracts may act on the same target, as for urease, and an interaction estimated against one reference model may not hold against another (Section 6.1). A combination is therefore treated as promising only if it passes under both the Bliss and the Loewe-based criteria.

Clinical and epidemiological evidence would be needed to determine whether *H. pylori* eradication and reduced chronic gastritis alter future gastric-cancer risk. The proposed combination has not been shown to prevent gastric cancer, and molecular docking cannot resolve this question.

Future Research Directions

Future work should begin by testing the three axes experimentally. The identity and concentration of marker compounds should first be confirmed in standardized preparations. Subsequent studies can assess urease inhibition, bacterial growth, biofilm formation, adhesion, and virulence-associated outcomes. Gastric epithelial models can then be used to investigate inflammatory signalling, oxidative stress, barrier integrity, autophagy, and cytotoxicity. Molecular dynamics may complement docking by examining the stability of predicted compound–target interactions.

Once single-agent data are available, the model in Section 6 can be fitted and evaluated. If it cannot reproduce the single-agent concentration–response curves, it should be revised before combination results are interpreted. The next step is to determine whether the proposed interactions are complementary in practice. Checkerboard assays, FIC analysis, and concentration–response modelling can be used to classify combinations as synergistic, additive, indifferent, or antagonistic. Establishing which outcome occurs is the central experimental question.

CONCLUSION

This conceptual framework proposes that bioactive constituents of *Nigella sativa* and *Punica granatum*, particularly pomegranate peel, may have complementary effects against *H. pylori* across three axes: bacterial survival and persistence, virulence and host interaction, and host and mucosal injury. *N. sativa* has been associated with potential mechanisms including urease inhibition, antibiofilm activity, effects on virulence-associated targets, increased mucin, modulation of gastric acid secretion, and inflammatory regulation. *P. granatum* peel has been investigated for antibacterial and membrane-related effects, urease inhibition, antioxidant and anti-inflammatory activity, autophagy-related responses, and mucosal protection. These mechanisms provide a rationale for further study, but their combined effects remain to be established.

The framework does not claim that the two plants are synergistic or that they directly prevent gastric cancer. Instead, it expresses the proposed interaction through an endpoint-specific parameter that can be estimated experimentally. Docking, ADMET analysis, molecular dynamics, and network pharmacology may help generate and prioritize hypotheses, but microbiological, biochemical, cell-based, and in vivo studies are needed to test them.

The central proposition is that selected constituents of *N. sativa* and *P. granatum* warrant investigation for possible complementary, multi-target activity against *H. pylori*. Gastric carcinogenesis is included only as downstream biological context, not as a demonstrated therapeutic target.

ETHICS APPROVAL

This conceptual study did not involve human participants, human data, or animal tissues or specimens. Ethics committee approval was therefore not applicable.

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COMPETING INTERESTS

The authors declare no conflict of interest.

DATA AVAILABILITY

No new experimental data were generated or analyzed in this conceptual study. Data sharing is therefore not applicable.

CONSENT TO PUBLISH

Not applicable. This study did not involve human participants, human data, or human tissue.

CONSENT TO PARTICIPATE

Not applicable.

AUTHOR CONTRIBUTIONS

- Mubarak Umar: Conceptualization, investigation, and writing original draft
- Abdulmajid Aliyu Abdullahi: Manuscript review
- Umesh Garu: Figure preparation and review
- Abba Sani: Editing
- Uzairu Bala: Manuscript review
- Pramod Mehta: Mathematical formulation of the proposed model.
- Habu Badamasi: Review
- Jamilu Rilwan Ahmad: Model editing
- Ahmad Sadi Shitu: Terminology check
- Aysha Manihar: Cross checking and observation
- Pradeep Singh: Review check

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