Original Article

JHSMR Journal of Health Science and Medical Research

Exploring the Synergistic Anti-inflammatory Properties of Clove Oil and Mangosteen Extract in LPS-Activated RAW 264.7 Macrophages

Nattakan Dangmanee, Ph.D.[2], Thunyaporn Ravangnam, M.Eng.[1], Chiramet Auranwiwat, Ph.D.[1], Kanyanat Kaewiad, Ph.D.[1]

[1]Expert Center of Innovative Herbal Products, Thailand Institute of Scientific and Technological Research (TISTR), Khlong Luang, Pathum Thani 12120, Thailand.

[2]Faculty of Agro and Bio Industry, Thaksin University, Pa Phayom, Phatthalung 93210, Thailand.

Received 8 May 2026 ● Revised 8 May 2026 ● Accepted 10 May 2026 ● Published online 17 September 2026

Abstract:

Objective: This study aimed to optimize and evaluate the synergistic anti-inflammatory effects of mangosteen extract and clove oil by assessing their inhibition of tumor necrosis factor-alpha (TNF-α) in lipopolysaccharide (LPS)-stimulated macrophages using the Response Surface Methodology (RSM).

Material and Methods: RAW 264.7 macrophages stimulated with LPS were used as an *in vitro* inflammation model. Central Composite Design (CCD) within RSM was employed to optimize the concentrations of mangosteen extract and clove oil for maximal inhibitory activity.

Results: Mangosteen extract and clove oil exhibited (IC_{50}) values of 2.37±1.12 mg/mL and 6.97±2.13 mg/mL, respectively, and showed dose-dependent inhibition of TNF-α production. The optimized combination (0.60 mg/mL mangosteen extract and 0.48 mg/mL clove oil) demonstrated a synergistic effect, achieving maximum inhibition of 43.30±1.4%.

Conclusion: These findings provide strong evidence for the synergistic anti-inflammatory potential of mangosteen extract and clove oil, supporting their development as natural alternatives for anti-inflammatory applications.

Keywords: anti-inflammatory, clove oil, mangosteen extract, RAW 264.7 cells, response surface methodology, tumour necrosis factor-α

This paper was from The 8th Current Drug Development International Conference 2026 (May 21-23, 2026).
Contact: Kanyanat Kaewiad, Ph.D.
Expert Center of Innovative Herbal Products, Thailand Institute of Scientific and Technological Research (TISTR), Khlong Luang, Pathum Thani 12120, Thailand.
E-mail: Kanyanat@tistr.or.th

J Health Sci Med Res
doi: 10.31584/jhsmr.20261426
www.jhsmr.org

Introduction

Inflammation is a fundamental innate immune response that protects the body against tissue damage, infection, and harmful stimuli. Lipopolysaccharide (LPS) acts as a potent inflammatory trigger by activating macrophages and initiating signaling pathways that lead to the production of pro-inflammatory mediators, including tumor necrosis factor-α (TNF-a),, interleukin-1, interferons, prostaglandins, and nitric oxide. TNF-α, a key cytokine produced by activated macrophages, plays a crucial role in mediating apoptosis in tumor cells and pathogen- infected cells[1,2].

Anti-inflammatory activity is critical, as numerous diseases are associated with dysregulated inflammatory processes. Inflammation is a fundamental component of the immune system and host defense against infections and foreign agents. External stimuli activate signaling pathways, including mitogen-activated protein kinases (MAPK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and activator protein (AP)-1, leading to increased vascular permeability, leukocyte infiltration, and the production of reactive oxygen species (ROS) and inflammatory mediators, ultimately contributing to tissue damage[3-5].

Natural products have long contributed to the development of therapeutic agents for various human diseases[6]. Tropical plants such as *Garcinia mangostana* L. (mangosteen) and *Syzygium aromaticum* (L.) Merr. & Perry (Myrtaceae) are well recognized for their diverse pharmacological properties.

G. mangostana Linn., locally known in Thailand as "Mangkhut" and commonly referred to as the "Queen of Fruits," belongs to the Guttiferae family[7]. The fruit pericarp is rich in bioactive xanthones, with α-mangostin and γ-mangostin being the most abundant xanthones in the pericarp of mangosteen fruit[8-10]. Notably, α-mangostin has been shown to exert anti-inflammatory effects by suppressing NF-κB and MAPK signaling pathways, thereby inhibiting TNF-α and IL-6 production[3].

Cloves, the dried flower buds of *S. aromaticum* (L.) Merr. & Perry (Myrtaceae), have been traditionally used to alleviate toothaches and oral or throat inflammation[11]. Numerous studies have confirmed their antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, and immunomodulatory activities[12,13]. Notably, clove extract has been shown to inhibit the production of inflammatory mediators, including nitric oxide (NO), prostaglandin E_2 (PGE_2), interleukin-6 (IL-6), interleukin-1β (IL-1β), and interleukin-8 (IL-8), in LPS-stimulated macrophages[14,15].

Conventional optimization methods, such as the one-factor-at-a-time approach, are time-consuming and fail to account for interactions between variables, potentially leading to suboptimal conclusions. To address these limitations, the Response Surface Methodology (RSM) was selected as a robust statistical and mathematical approach to model complex interactions and optimize formulation conditions using multivariate polynomial functions.

Synergistic interactions among anti-inflammatory agents offer significant therapeutic advantages, including enhanced efficacy, reduced dosage requirements, and minimized toxicity or adverse effects. In the present study, a modified and optimized assay was employed to evaluate the combined anti-inflammatory activity of mangosteen extract and clove oil, with the aim of identifying low-concentration combinations that exhibit synergistic effects. These findings may support the development of safe and effective natural anti-inflammatory formulations as alternatives to synthetic agents.

To the best of our knowledge, this study is the first to investigate the synergistic anti-inflammatory effects of mangosteen extract and clove oil in LPS-stimulated RAW 264.7 macrophage cells.

Material and Methods

Chemicals and reagents

Dulbecco's Modified Eagle Medium (DMEM), penicillin–streptomycin, fetal bovine serum (FBS), and phosphate-buffered saline (PBS) were obtained from Gibco-Life Technologies LLC. (Rockville, MD, USA). LPS derived from *E. coli* (0111: B4), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and Human TNF-α ELISA kits were purchased from Sigma-Aldrich (St. Louis, MO, USA). α-Mangostin and eugenol reference standards were obtained from TCI (Japan) and Biopurify (China), respectively. All solvents, including formic acid, acetonitrile, and methanol, were of HPLC grade and purchased from RCI Labscan (Thailand).

Extraction of *Garcinia mangostana* peels and clove buds

Dried *G. mangostana* peels were extracted with ethanol using ultrasonic-assisted extraction (UAE) (30 min per cycle, three cycles). The combined extracts were filtered and concentrated under reduced pressure to obtain the crude *G. mangostana* extract. Clove buds were extracted by steam distillation for 6 h, and the resulting distillate was partitioned with dichloromethane and dried over anhydrous Na_2SO_4 to yield clove oil as a brown liquid.

HPLC determination of α-mangosteen in *Garcinia mangostana* peel extract

Quantification of α-mangostin was performed using an HPLC system (ACQUITY Arc UHPLC, Waters, USA) equipped with a Kromasil 100-5-C18 column (4.6x250 mm, 5 µm). Chromatographic separation was achieved with a flow rate of 1.0 mL/min, an injection volume of 20 µL, and ā detection at 320 nm. The mobile phase consisted of 0.1% formic acid (A) and acetonitrile (B), using the following gradient program: 0-10 min, 350% A; 10-15 min, 3.0% A; 15-16 min, constant at 3.0% A; 16-20.0%, 35.0% A; and 20-30 min, constant at 35.0%A. Standard α-mangostin solutions (3.125-100 µg/mL) were used to construct the calibration curve. *G. mangostana* extract was prepared at 100 µg/mL, filtered through a 0.22 µm syringe filter, and injected into HPLC. Quantification was performed by comparing the peak area of the sample with that of the standard. The Ultra High Performance Liquid Chromatography (UHPLC) was performed on Arc UHPLC Water.

GC determination of eugenol in clove oil

Eugenol content in clove oil was quantified using gas chromatography with flame ionization detection (GC-FID) (Agilent Technologies 7890, USA) equipped with a DB-5 capillary column (0.25 mm x 30 m, 0.25 µm). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injection volume was 1.0 µL with a split ratio of 1:10. The inlet and detector temperatures were maintained at 180 °C and 290 °C, respectively. The oven temperature program was set from 60 °C to 175 °C at 3 °C/min, followed by a ramp to 270 °C at a rate of 70 °C/min, and post run at 280 °C for 10 min. Standard eugenol solutions (50-500 µg/mL) were used to construct the calibration curve. Clove oil samples were prepared at 10 mg/mL and analyzed under the same conditions. Quantification was performed by comparing the peak area of the sample with that of the standard.

Cell culture

RAW 264.7 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10.0% heat-inactivated fetal bovine serum (FBS), 100 units/mL of penicillin, and 0.1 mg/ mL of streptomycin. Cells were maintained at 37 °C in a humidified

atmosphere of 5.0% CO_2 and 95.0% air. Mangosteen extract and clove oil were dissolved in dimethyl sulfoxide (DMSO), and the final DMSO concentration was maintained at 0.1% (v/v) in all experimental conditions.

Cell viability assay

Cell viability was determined using the MTT assay as previously described[17]. RAW 264.7 cells were seeded in 96-well plates at a density of 50,000 cells/well and incubated for 24 h to allow cell attachment. Cells were then treated with serial concentrations of test compounds in complete medium for 24 h. Following treatment, the medium was replaced with 100 µL of fresh medium containing 0.1 mg/mL MTT and incubated for 2 h at 37 °C in a humidified atmosphere of 5.0% CO_2. The MTT-containing medium was removed, and the resulting formazan crystals were dissolved in 100 µL of DMSO. Absorbance was measured at 570 nm using a microplate reader (Infinite®M NANO, Tecan, Austria).

Measurement of TNF-α accumulation

RAW 264.7 murine macrophage cells were seeded in 96-well plates at a density of (5×10^4 cells/well) and incubated for 24 h. Cells were then stimulated with lipopolysaccharide (LPS, 1 µg/mL) in the presence or absence of serial concentrations of test compounds for 24 h. Following incubation, culture supernatants were collected, and TNF-α levels were quantified using a commercial ELISA kit; according to the manufacturer's instructions.

Experimental design

Optimization of the formulation was carried out using response surface methodology (RSM) with a central composite design (CCD). The independent variables were the concentrations of mangosteen extract (X_1) and clove oil (X_2). The experimental ranges and center point values for each variable are provided in Table 1.

The combination variables were coded according to the following equation:

$$X=(Xi-X0)/\Delta X$$

where x is the dimensionless coded value, X_i is the actual value of variables, X_0 is the actual value of variables at the centre point, and ΔX is the step change value. After the conduct of the experiment, the data were fitted with a second-order polynomial equation as follows:

$$Y= \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2$$

To set up a regression model, we used Y to denote TNF-α inhibition, (%), which is calculated by the model, and determined coded factor levels as follows: X_1=mangosteen extract; X_2=clove oil. β_0 is a constant, β_1 and β_2 are linear, β_{11} and β_{22} are squared, and β_{12} is the interaction coefficient.

Data analysis

Experimental design, regression analysis, and response prediction were performed using Design-Expert software (Version 13.0). Statistical differences between groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with statistical significance set at p-value<0.05.

Table 1 Independent variables and their coded and actual values used for optimization

Independent variables	Units	Symbol	Coded levels				
			-α	-1	0	1	+α
Mangosteen extract	mg/mL	X_1	0.03	0.2	0.6	1	1.16
Clove oil	mg/mL	X_2	0.005	0.15	0.5	0.85	0.99

Verification of the model

Numerical optimization was conducted to determine the optimal conditions for maximizing TNF-alpha inhibition (%), based on the developed regression model and three-dimensional (3D) response surface plots of the independent variables. The predicted optimal conditions were experimentally validated, and the observed responses were compared with the predicted values to assess the accuracy and reliability of the model.

Results

HPLC profiling of α-mangostin and eugenol

Ethanolic extraction of *Garcinia mangostana* peels yielded 25.6% (w/w) crude extract. The calibration curve for α-mangostin demonstrated excellent linearity (r^2=0.9996), and its content in the extract was quantified as 20.9% (w/w). Steam distillation of clove buds produced clove oil with a yield of 12.4% (v/w). Based on the eugenol calibration curve (r^2=0.9995), the eugenol content in the clove oil was determined to be 87.9%.

Effects of mangosteen extract and clove oil on RAW 264.7 cell viability

The cytotoxic effects of mangosteen extract and clove oil on RAW 264.7 cells were evaluated using the MTT assay to establish non-toxic concentration ranges. As shown in Figure 1A-B, mangosteen extract (up to 2 mg/mL) and clove oil (up to 1 mg/mL) did not cause any significant reduction in cell viability.

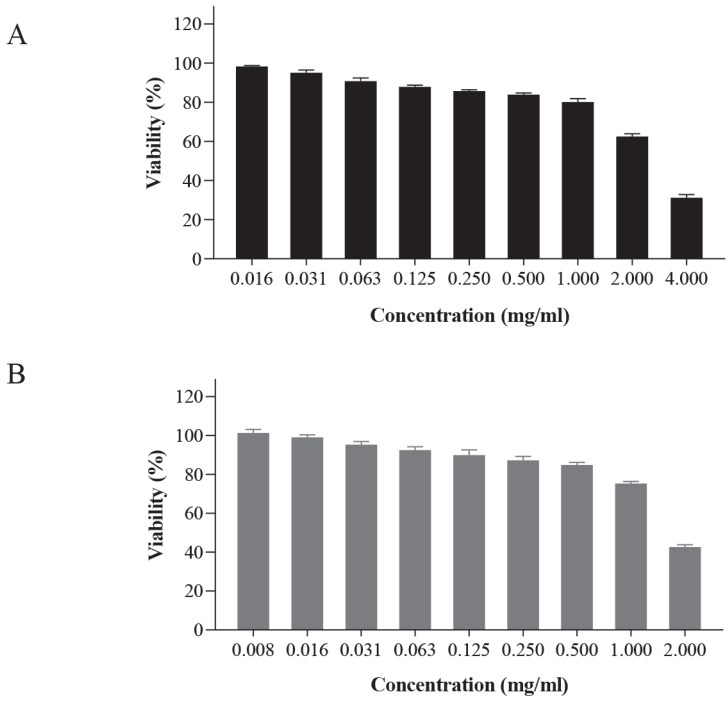


Figure 1 Cytotoxicity profiles of mangosteen extract (A) and clove oil (B) in RAW 264.7 macrophage cells

Effects of mangosteen extract and clove oil on TNF-α production in LPS-stimulated RAW 264.7 cells

Using the established non-cytotoxic concentration ranges, the inhibitory effects of mangosteen extract and clove oil on TNF-α production were evaluated in LPS-stimulated RAW 264.7 cells. LPS stimulation of RAW 264.7 macrophage cells for 24 h significantly increased TNF-α secretion to 88.77±4.88 pg/mL. Following treatment with mangosteen extract and clove oil, TNF-α production was significantly reduced in a dose-dependent manner (Table 2). The positive control (D,L-1'-Acetoxychavicol acetate) exhibited a high inhibition rate (53.03±2.58%), confirming the sensitivity and reliability of the assay. Mangosteen extract demonstrated dose-dependent inhibition, with inhibition percentages of 18.79±2.17%, 22.29±1.39%, and 30.81±3.12% at 0.200, 0.500, and 1.000 mg/mL, respectively. Similarly, clove oil exhibited concentration-dependent inhibition, with inhibition percentages of 12.36±2.75%, 19.23±3.59%, and 24.88±1.63% at 0.150, 0.400, and 0.800 mg/mL, respectively.

Central composite design results

A two-factor inscribed central composite design (CCD) was employed to evaluate the relationship between the independent variables and the response, and to optimize the combination for maximum anti-inflammatory activity.

Inflammation is a complex and multifactorial process, and single-agent therapies may be insufficient to achieve optimal therapeutic outcomes. Moreover, the high-dose administration of individual agents may lead to undesirable toxicity. Growing evidence indicates that combination strategies targeting multiple molecular pathways can produce synergistic interactions, resulting in enhanced biological activity[18]. Such synergism may improve the therapeutic efficacy while reducing the required dosages and minimizing potential adverse effects.

The TNF-α inhibition (%) ranged from 15.9% to 44.9% under the tested experimental conditions. Based on these results, the combination parameters were optimized to achieve maximum anti-inflammatory activity.

Table 2 Effects of mangosteen extract and clove oil on TNF-α production levels and percentage inhibition in LPS-stimulated RAW 264.7 macrophages

Treatment	Concentration	TNF- α production (pg/mL)	% Inhibition of TNF- α production
Control	-	29.95±4.14	-
Negative control:			
Cell induced with LPS (mg/mL)	0.01	88.77±4.88	
Positive control:			
D,L-1'-Acetoxychavicol Acetate (mg/mL)	0.10	41.37±3.37	53.03±2.58
Mangosteen extract (mg/mL)	0.20	71.89±1.44	18.79±2.17
	0.50	68.82±1.58	22.29±1.39
	1.00	61.27±1.42	30.81±3.12
Clove oil (mg/mL)	0.15	77.53±1.60	12.36±2.75
	0.40	71.52±1.00	19.23±3.59
	0.80	66.46±0.35	24.88±1.63

LPS=lipopolysaccharide, mg=miligram, mL=millimeter

Fitting the model

The experimental data were fitted to a second-order polynomial model, and the results are summarized in Table 3. Analysis of variance (ANOVA) indicated that the model was statistically significant (p-value<0.05), with significant regression coefficients identified based on the F-test. A strong agreement between predicted and experimental values confirmed the adequacy of the model. The model exhibited a high coefficient of determination (R^2=0.9735) and an adjusted R^2 exceeding 0.90, indicating excellent predictive capability. The lack-of-fit test was not significant (p-value>0.05), confirming good model fitness. The predicted quadratic model in terms of coded variables is presented in Table 4.

Effect of combination conditions on anti-inflammatory activity

The anti-inflammatory activity was significantly influenced by the concentrations of mangosteen extract and clove oil. The significance of each factor was evaluated using the coefficients of the second-order polynomial regression model. The results showed that the linear terms (X_1 and X_2) were not statistically significant (p-value>0.05), whereas the quadratic terms (X_1 and X_2) and the interaction term (X_1X_2) were statistically significant (p-value<0.05). These findings indicate a non-linear response surface with significant interaction effects between the two variables.

Table 3 Central composite design (CCD) matrix and corresponding experimental and predicted values for TNF-α inhibition (%)

Standard order	Run order	Coded variables		Response (Y1)	
		Mangosteen extract	Clove oil	Experimental	Predicted
5	1	0.0343146	0.5	20.56±0.45	22.03
13	2	0.6	0.5	42.18±0.12	43.29
9	3	0.6	0.5	42.31±0.83	43.29
8	4	0.6	0.994975	21.54±0.25	20.45
6	5	1.16569	0.5	22.23±0.54	23.17
2	6	1	0.15	21.09±0.21	18.43
3	7	0.2	0.85	15.93±0.67	16.15
10	8	0.6	0.5	44.89±0.39	43.29
7	9	0.6	0.00502525	19.02±0.22	22.53
4	10	1	0.85	25.87±0.30	26.47
1	11	0.2	0.15	30.16±0.16	27.13
11	12	0.6	0.5	44.64±0.44	43.29
12	13	0.6	0.5	42.46±0.56	43.29

TNF-α=tumor necrosis factor-α

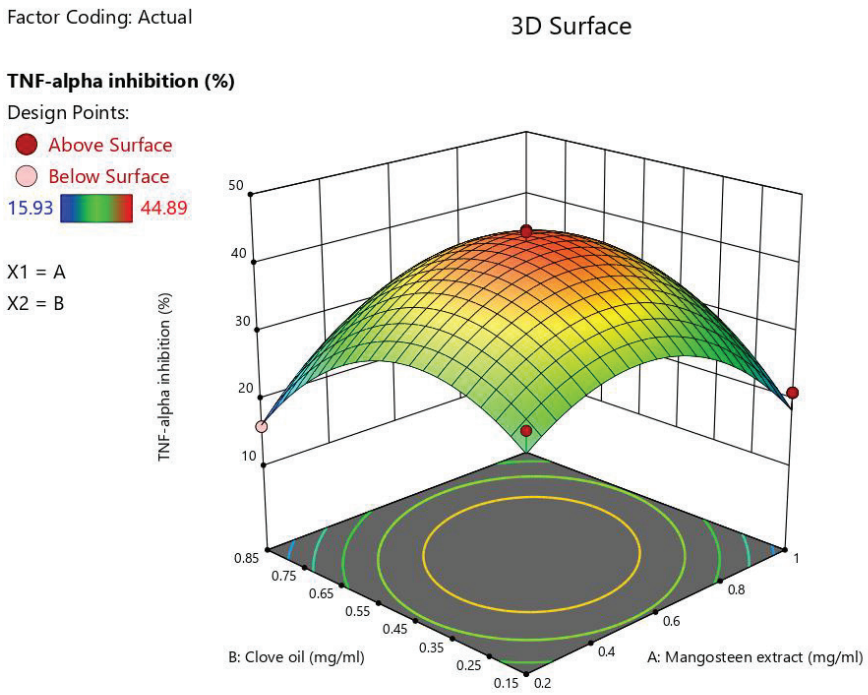
Table 4 The fitted quadratic model in terms of coded variables for Y response

Response	2nd Order polynomial equation	Regression (p-value)	R^2	R^2 (adjusted)	Lack of fit
Y	$8.40205 + 61.6186(X_1) + 66.5238(X_2) + 33.9464(X_1)(X_2) - 64.6516(X_1)^2 - 88.9939(X_1)^2$	0.0001	0.9735	0.9546	0.0566

The interaction term (X_1X_2) was statistically significant (p-value<0.05), indicating a strong interaction between mangosteen extract and clove oil in influencing TNF-α inhibition. The 3D response surface plot (Figure 2) revealed that anti-inflammatory activity increased with both variables at intermediate concentrations, followed by a decline at higher levels, suggesting a non-linear (quadratic) effect. The normal probability plot of studentized residuals (Figure 3) demonstrated that the residuals were normally distributed without significant deviation, confirming model validity. The high coefficient of determination (R^2>0.9735) further supported the goodness-of-fit of the model. Notably, the combined treatment exhibited greater inhibitory effects on TNF-α production in LPS-stimulated macrophages compared to individual treatments, even at higher concentrations, indicating a synergistic interaction.

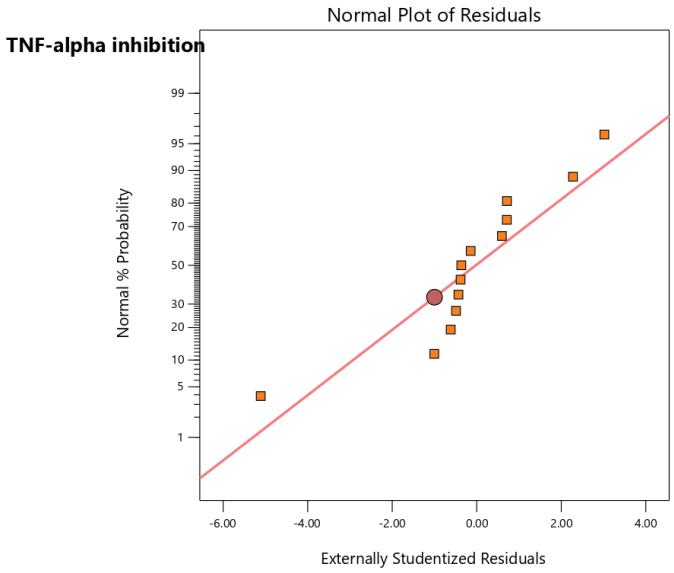
Optimization of responses and model validation

Numerical optimization of extraction conditions was performed using Design-Expert software, based on the preliminary experimental results, with the aim of maximizing anti-inflammatory activity. The optimal conditions were identified as 0.60 mg/mL mangosteen and 0.48 mg/mL clove oil. Model adequacy was assessed by comparing predicted and experimental responses (Table 5). The experimental results closely matched the predicted values, with no statistically significant deviation (p-value>0.05).



TNF-α=tumor necrosis factor-α

Figure 2 Three-dimensional response surface plot illustrating the interaction between mangosteen extract (X_1) and clove oil (X_2) concentrations on TNF-α inhibition (%)



TNF-α=tumor necrosis factor-α

Figure 3 Normal probability plot of studentized residuals corresponding to the maximum TNF-α inhibition (%)

Table 5 Comparison of predicted and experimental responses for model validation of optimized parameter combinations

	Predicted value	Experimental value[a]
Y (%)	43.30	44.96±1.53

[a]Values are expressed as the mean of triplicate determinations

Discussion

Chemical analysis confirmed that the extracts were rich in bioactive markers, particularly α-mangostin (20.9% w/w) and eugenol (87.9%). This combination is expected to contribute synergistically to anti-inflammatory activity. MTT assays performed prior to biological evaluation showed that mangosteen (≤2 mg/mL) and clove oil (≤1 mg/mL) were non-cytotoxic to RAW 264.7 cells, indicating that TNF-α inhibition was not mediated by cytotoxicity.

In the inflammatory response model, LPS treatment significantly increased TNF-α secretion by macrophages. TNF-α, a key pro-inflammatory mediator, induces iNOS transcription through NF-κB activation, which binds to κB response elements in the NOS promoter[19,20]. Moreover, elevated pro-inflammatory cytokine levels have been linked to tumor growth and disease progression[21].

Non-cytotoxic levels of α-mangostin, isolated from *Garcinia mangostana*, significantly reduce TNF-α secretion in various human cell models. This suggests that its anti-inflammatory effects may involve biotransformation into other active xanthone metabolites. Furthermore, α- and γ-mangostins attenuate *P. acnes*-induced expression of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, in keratinocytes via inhibition of NF-κB and MAPK signaling pathways[22].

Syzgium aromaticum and its principal constituent eugenol have been shown to attenuate cytokine production in lipopolysaccharide (LPS)-stimulated murine RAW

264.7 macrophage cells[23]. Eugenol, the dominant bioactive compound in clove extract and essential oil, is largely responsible for these anti-inflammatory effects, including suppression of COX-2 expression in activated macrophages[24]. *In vitro* studies further demonstrate that clove extract and eugenol regulate the production of IL-1β, IL-6, and IL-10 in murine macrophages[25]. Overall, clove reduces LPS-induced secretion of TNF-α, IL-1β, IL-6, and IL-10, likely through eugenol-mediated inhibition of the signaling pathway.

The combined use of mangosteen and clove oil was designed to achieve a multi-target anti-inflammatory strategy. α-Mangostin effectively suppresses TNF-α production and MAPK signaling, whereas eugenol inhibits COX-2 expression and PGE_2 synthesis via the NF-κB cascade. The present findings demonstrate a synergistic interaction, whereby the dual-extract system exhibits superior anti-inflammatory efficacy at lower, non-cytotoxic concentrations compared with single-extract treatments. This synergy is likely driven by complementary modulation of NF-κB, MAPK, and COX-2 inflammatory pathways.

Furthermore, the Response Surface Methodology (RSM) using Central Composite Design (CCD) proved to be a robust approach for modelling the interaction between mangosteen and clove oil, yielding a highly significant second-order model (R^2=0.9735, p-value<0.0001). A significant interaction effect (X_1X_2) was observed, whereby the combined treatment enhanced TNF-α inhibition to 43.3%, exceeding that of the individual extracts. This synergistic effect is likely driven by the complementary regulation of NF-κB, MAPK, and COX-2 pathways by α-mangostin and eugenol. The optimized formulation (0.60 mg/mL mangosteen and 0.48 mg/mL clove oil) represents a potent, low-toxicity natural anti-inflammatory candidate.

Conclusion

The influence of mangosteen and clove oil concentrations on anti-inflammatory activity was successfully optimized using the Response Surface Methodology. Both factors significantly contributed to the response. The optimal formulation (0.60 mg/mL mangosteen and 0.48 mg/mL clove oil) achieved a predicted TNF-α inhibition of 43.3%. Overall, the dual-extract system demonstrated synergistic inhibition of LPS-induced inflammation in RAW 264.7 macrophages, as reflected by reduced TNF-alpha levels. The effect observed in this study was associated with synergistic interactions between α-mangostins and eugenol in the combined mangosteen + clove oil cotreatment. These findings provide novel insights into the interactions among bioactive compounds, an area that remains insufficiently explored. The results may support the development of bioactive-enriched functional food systems designed to manage inflammation-related diseases through underlying biochemical and physiological pathways.

Acknowledgement

The authors are grateful to the Thailand Institute of Scientific and Technological Research for its technical support and access to research facilities.

Funding sources

The Thailand Institute of Scientific and Technological Research funded this study.

Conflict of interest

The authors report no conflicts of interest in this work.

References

1. Standiford T. Anti-inflammatory cytokines and cytokine antagonists. Curr Pharm Design 2000;6:633–49.

2. Orlicek SL, Meals E, English BK. Differential effects of tyrosine kinase inhibitors on tumor necrosis factor and nitric oxide production by murine macrophages. J Infect Dis 1996;174; 638–42.
3. Bumrungpert A, Kalpravidh RW, Chuang CC, Overman A, Martinez K, Kennedy A, et al. Xanthones from mangosteen inhibit inflammation in human macrophages and in human adipocytes exposed to macrophage-conditioned media. J Nutr 2010;140:842-7.
4. Feng Z, Lu X, Gan L, Zhang Q, Lin L. Xanthones, a promising anti-inflammatory scaffold: Structure, activity, and drug likeness analysis. Molecules 2020;25:598.
5. Widowati W, Darsono L, Suherman J, Fauziah N, Maesaroh M, Erawijantari PP. Anti-inflammatory effect of mangosteen (*Garcinia mangostana* L.) peel extract and its compounds in LPS-induced RAW 264.7 cells. Nat Prod Sci 2016;22:147-53.
6. Cragg GM, David JN, Kenneth MS. Natural products in drug discovery and development. J Nat Prod 1997;60:52-60.
7. Farnsworth NR, Bunyapraphatsara N. Thai Medicinal Plants. Bangkok: Prachachon Co., Ltd.; 1992.
8. Pedraza-Chaverri J, Cardenas-Rodríguez N, Orozco-Ibarra M, Perez-Rojas JM. Medicinal properties of mangosteen (*Garcinia mangostana*). Food Chem Toxicol 2008;46:3227–39.
9. Chaivisuthangkura A, Malaikaew Y, Chaovanalikit A, Jaratrungtawee A. Prenylated xanthone composition of *Garcinia mangostana* (mangosteen) fruit hull. Chromatographia 2009;69:315–18.
10. Walker EB. HPLC analysis of selected xanthones in mangosteen fruit. J Sep Sci 2007;30:1229–34.
11. Debjit B, Sampath Kumar KP, Akhilesh Y, Shweta S, Shravan P, Amit Sankar D. Recent trends in Indian traditional herbs *Syzygium aromaticum* and its health benefits. J Pharmacogn Phytochem 2012;1:13–22.
12. Lopes FC, Silva LA, Tichota DM, Daroit DJ, Velho RV, Pereira JQ, et al. Production of Proteolytic Enzymes by a Keratin-Degrading *Aspergillus niger*. Microbiol 2011;42:1479–84.
13. Johannah NM, Renny RM, Gopakumar G, Balu Maliakel, Sureshkumar D, Krishnakumar IM. Beyond the flavour: a de-flavoured polyphenol rich extract of clove buds (*Syzygium aromaticum* L) as a novel dietary antioxidant ingredient. Food Funct 2015;610:73-82.
14. Daneshmandi S, Dibazar SP, Fateh S. Effects of 3-dimensional culture conditions (collagen-chitosan nano-scaffolds) on maturation of dendritic cells and their capacity to interact with T-lymphocytes. J Immunotoxicol 2016;13;235–42.
15. Bachiega TF, de Sousa JPB, Bastos JK, Sforcin JM. Clove and eugenol in noncytotoxic concentrations exert immunomodulatory/anti-inflammatory action on cytokine production by murine macrophages. J Pharm Pharmacol 2012;64:610–6.
16. Yolmeh M, Jafari SM. Applications of Response Surface Methodology in the Food Industry 427 Processes. Food Bioprocess Tech 2017;10:413–33.
17. Nuamnaichati N, Mangmool S, Chattipakorn N, Parichatikanond W. Stimulation of GLP-1 receptor inhibits methylglyoxal-induced mitochondrial dysfunctions in H9c2 cardiomyoblasts: potential role of Epac/PI3K/Akt pathway. Frontiers in pharmacology 2020;11:805.
18. Xiao H, Yang CS. Combination regimen with statins and NSAIDs: a promising strategy for cancer chemoprevention. Int J Cancer 2008;123:983–90.
19. Hibbs JBJr. Synthesis of nitric oxide from L-arginine: a recently discovered pathway induced by cytokines with antitumour and antimicrobial activity. Res Immunol 1991;142:565–98.
20. Dinarello CA. The paradox of pro-inflammatory cytokines in cancer. Cancer Metastasis Rev 2006;25:307–13.
21. Lin WW, Karin MA. cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest 2007;117:1175–83.
22. Bumrungpert A, Kalpravidh RW, Chitchumroonchokchai C, Chuang CC, West T, Kennedy A, et al. Xanthones from mangosteen prevent lipopolysaccharide - mediated inflammation and insulin resistance in primary cultures of human adipocytes. J Nutr 2009;139:1185–91.
23. Setiawan AA, Budiman J, Prasetyo A. Anti-inflammatory potency of mangosteen (*Garcinia mangostana* L.): a systematic review. Open Access Maced J Med Sci 2023;11:58-66.
24. Kim SS, Oh OJ, Min HY, Park EJ, Kim Y, Park HJ, et al. Eugenol Suppresses Cyclooxygenase - 2 Expression in Lipopolysaccharide - stimulated Mouse Macrophage RAW 264.7 Cells. Life Sci 2003;73:337–48.
25. Peng C, Feng Z, Ou L, Zou Y, Sang S, Liu H, et al. *Syzygium aromaticum* enhances innate immunity by triggering macrophage M1 polarization and alleviates Helicobacter pylori-induced inflammation. J Funct Foods 2023;107;105626.