Research Article ISSN 2641-4295

Food Science & Nutrition Research

Plant-Derived Methyl-Eugenol as a Selective Modulator of Platelet Function and a Potential Cardiovascular Protective Agent

Yi Chang[1,2,3], Joen-Rong Sheu[2], Chih-Wei Hsia[4] and Wei-Chieh Huang[2]*

[1]*Department of Anesthesiology, Shin Kong Wu Ho-Su Memorial Hospital, Taipei 111, Taiwan.*

[2]*Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, Taipei 110, Taiwan.*

[3]*School of Medicine, College of Medicine, Fu Jen Catholic University, New Taipei City 242, Taiwan.*

[4]*Department of Medical Research, Taipei Medical University Hospital, Taipei 110, Taiwan.*

***Correspondence:**
Dr. Wei-Chieh Huang, Graduate Institute of Medical Sciences, College of Medicine, Taipei Medical University, 250 Wu-Hsing St., Taipei 11031, Taiwan. Tel: +886-2-27361661 ext. 3192.

Received: 21 Jul 2025; **Accepted:** 27 Aug 2025; **Published:** 05 Sep 2025

Citation: Yi Chang, Joen-Rong Sheu, Chih-Wei Hsia, et al. Plant-Derived Methyl-Eugenol as a Selective Modulator of Platelet Function and a Potential Cardiovascular Protective Agent. Food Sci Nutr Res. 2025; 8(3): 1-5.

ABSTRACT

Cardiovascular diseases remain the leading cause of death worldwide, with arterial thrombosis representing a central mechanism. Platelets, though anucleate, coordinate adhesion, aggregation, and secretion events that stabilize thrombus formation. Collagen- and von Willebrand factor–mediated adhesion activates intracellular pathways, culminating in integrin $\alpha IIb\beta_3$ activation, granule release, and thromboxane A_2 synthesis. While current antiplatelet agents are clinically important, their limitations highlight the need for alternative strategies. Methyl-eugenol is a naturally occurring constituent of various plants, including Croton malambo, Cinnamomum cordatum, and Melaleuca bracteata; however, its role in platelet activation remains unclear. This study, methyl-eugenol significantly inhibited collagen-induced aggregation in a dose- dependent manner (20–80 μM) without affecting thrombin-induced responses, indicating agonist selectivity. It also suppressed collagen-induced ATP release and markedly reduced P-selectin expression, reflecting inhibition of dense and α-granule secretion. These findings reveal that methyl-eugenol selectively attenuates collagen- mediated platelet activation, providing mechanistic insights and supporting its potential as a natural scaffold for future antithrombotic development.

Keywords

Human platelet, methyl-eugenol, ATP, platelet aggregation, P-selectin expression, collagen.

Introduction

Cardiovascular diseases continue to dominate as the foremost cause of death worldwide, with arterial thrombosis serving as a central pathological driver. The event typically originates when an atherosclerotic plaque ruptures or erodes, exposing thrombogenic components beneath the endothelium. Such exposure rapidly initiates platelet adhesion, aggregation, and activation of the coagulation cascade, culminating in arterial occlusion [1]. Depending on the site of occurrence, arterial thrombosis manifests as myocardial infarction in the coronary circulation, ischemic stroke in the cerebral vasculature, or peripheral artery disease in the limbs. Owing to its crucial role in disease onset and progression, arterial thrombosis remains a major focus of clinical management, with antiplatelet therapy positioned as a cornerstone strategy for prevention and treatment [2].

Platelets, though only 2–4 μm in diameter and lacking a nucleus, play an outsized role in hemostasis and thrombosis. Derived from megakaryocytes, these circulating fragments ordinarily remain inactive; yet, once vascular integrity is compromised, they are swiftly recruited to the injury site. Collagen and von Willebrand factor act as the primary triggers of platelet adhesion, initiating a series of intracellular signaling cascades [3]. Activated platelets undergo cytoskeletal reorganization, release dense and α-granule contents, and generate potent mediators such as adenosine diphosphate (ADP) and thromboxane A_2 (TXA_2), thereby

amplifying the recruitment of additional platelets.

Collagen-mediated signaling through receptors including glycoprotein VI (GPVI) and integrin $\alpha_2\beta_1$ engages pathways involving Src kinases, phospholipase Cγ2 (PLCγ2), and protein kinase C (PKC), ultimately converging on the activation of integrin $\alpha IIb\beta_3$ to stabilize thrombus formation. This tightly coordinated sequence underpins both physiological hemostasis and pathological thrombotic events [4,5].

Alongside conventional pharmacotherapy, natural products have long attracted interest as sources of bioactive compounds with therapeutic potential. Eugenol (Figure 1A), a major constituent of clove (*Syzygium aromaticum*) oil, exemplifies this category and is known for its wide-ranging biological activities, including analgesic, anti- inflammatory, antioxidant, and vasodilatory properties [6-7]. Intriguingly, eugenol has also been reported to suppress platelet activation and reduce thrombus formation [8]. Methyl-eugenol (Figure 1B), which shares structural similarity with eugenol, has been reported as a naturally occurring constituent of numerous plant species, notably *Croton malambo*, *Cinnamomum cordatum*, and *Melaleuca bracteata* [9,10]. It is broadly distributed across medicinal herbs and culinary spices, yet its effects on platelet physiology remain poorly characterized despite its structural similarity to eugenol.

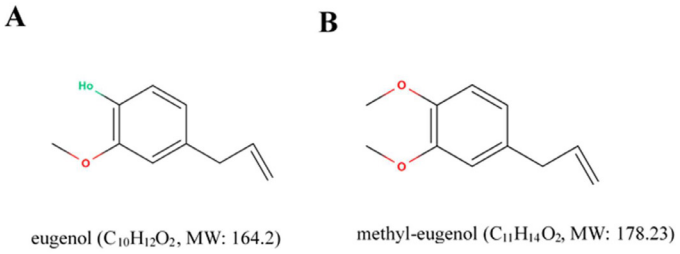

Figure 1: Chemical structures of (A) eugenol ($C_{10}H_{12}O_2$) and (B) methyl-eugenol ($C_{11}H_{14}O_2$).

To address this gap, the present study explores the capacity of methyl-eugenol to modulate human platelet activation and evaluates its potential as a candidate scaffold for future antithrombotic drug development. By dissecting its influence on collagen-induced platelet signaling and functional responses, this work aims to provide mechanistic insight into its biological activity and to establish a foundation for considering methyl-eugenol within the context of innovative therapeutic strategies for arterial thrombosis.

Methods

Chemicals and reagents

Methyl-eugenol (>98% purity) was obtained from MedChem Express (Monmouth Junction, NJ, USA). Reagents including luciferin–luciferase, dimethyl sulfoxide (DMSO), bovine serum albumin (BSA), ethylenediaminetetraacetic acid (EDTA), heparin, phenylmethylsulfonyl fluoride, sodium orthovanadate, sodium pyrophosphate, aprotinin, leupeptin, and sodium fluoride were purchased from Sigma-Aldrich (St. Louis, MO, USA). The protein assay dye reagent concentrate was obtained from Bio- Rad Laboratories (Hercules, CA, USA). Fluorescein isothiocyanate (FITC)-conjugated anti-human CD42P (P-selectin) monoclonal antibody was purchased from BioLegend (San Diego, CA, USA). Hybond-P polyvinylidene difluoride membranes, enhanced chemiluminescence Western blot detection reagents, horseradish peroxidase– conjugated donkey anti-rabbit immunoglobulin G (IgG), and sheep anti-mouse IgG were supplied by Amersham (Buckinghamshire, UK).

Preparation of human platelets and assessment of aggregatory function

This investigation was performed in compliance with the Declaration of Helsinki and received approval from the Institutional Review Board of Taipei Medical University. All volunteers provided written informed consent prior to participation. Peripheral blood was obtained from healthy adult donors who had refrained from taking any medication or substances known to interfere with platelet function for a minimum of 14 days before sampling. Blood was anticoagulated with acid–citrate–dextrose solution (9:1, v/v) and centrifuged to prepare platelet-rich plasma (PRP). PRP was subsequently treated with EDTA (2 mM) and heparin (6.4 IU/mL), followed by washing. The resulting platelet suspensions were resuspended in Tyrode's buffer containing 3.5 mg/mL BSA, and the calcium concentration was adjusted to 1 mM. Platelet aggregation was triggered using either collagen (2 μg/mL) or thrombin (0.04 U/mL), while 0.1% DMSO was employed as the vehicle control. For treatment groups, methyl-eugenol (20–80 μM) was added to the platelet suspension and incubated for 3 min prior to stimulation with agonists. Aggregation responses were measured with a Lumi- Aggregometer (Payton, Scarborough, Ontario, Canada).

Assessment of adenosine triphosphate (ATP) release

Dense granule secretion was determined by quantifying ATP release from activated platelets. In brief, 40 μL of luciferin–luciferase reagent was added directly to the platelet suspension 1 min prior to stimulation with collagen (2 μg/mL), allowing the enzymatic reaction to detect ATP liberated during platelet activation. Luminescence signals were continuously monitored and recorded using a fluorescence spectrophotometer (F-7000, Hitachi, Tokyo, Japan), providing a real-time assessment of dense granule secretion. For treatment groups, platelets were preincubated with methyl-eugenol (40 or 80 μM) for 3 min before collagen addition, whereas suspensions treated with 0.1% DMSO served as vehicle controls.

Platelet surface expression of P-selectin

Platelets were incubated with methyl-eugenol (40 or 80 μM) in combination with fluorescein isothiocyanate (FITC)-conjugated anti-P-selectin monoclonal antibody (2 μg/mL) for 3 min prior to stimulation. After the preincubation period, platelet activation was induced by the addition of collagen (2 μg/mL). Flow cytometric analysis was performed using a FACScan system (Becton

Dickinson, San Jose, CA, USA) to quantify fluorescein-labeled platelets. For each experimental condition, fluorescence signals were acquired from 50,000 platelets, which were identified and gated according to their characteristic forward- and side-scatter profiles. All experiments were repeated independently at least four times to ensure reproducibility of the measurements [11].

Statistical Analysis

Experimental results are expressed as the mean ± standard error of the mean (SEM). The value of n corresponds to the number of independent experiments performed with platelet samples obtained from separate healthy donors. Differences among multiple experimental groups were evaluated using one-way analysis of variance (ANOVA). When significant overall variance was observed, pairwise group comparisons were subsequently assessed with the Student–Newman–Keuls post hoc test to adjust for family-wise error. A probability value of $p < 0.05$ was predetermined as the criterion for statistical significance. All statistical analyses were conducted using SAS software (version 9.2; SAS Institute, Cary, NC, USA).

Result

Potent inhibition of collagen-induced platelet aggregation by methyl-eugenol Methyl-eugenol demonstrated a pronounced inhibitory effect on platelet aggregation when stimulated with collagen (2 µg/mL), with efficacy observed across the concentration range of 20–80 µM. This result indicates that methyl-eugenol is capable of attenuating collagen-mediated platelet activation in a dose-dependent manner. In contrast, when platelets were stimulated with thrombin (0.04 U/mL), methyl-eugenol did not exert any measurable inhibitory activity, suggesting that its antiplatelet effects are selective and may depend on the nature of the agonist involved (Figure 2). Taken together, these findings imply that methyl-eugenol modulates platelet responses through pathways preferentially engaged by collagen stimulation, while leaving thrombin-induced signaling largely unaffected.

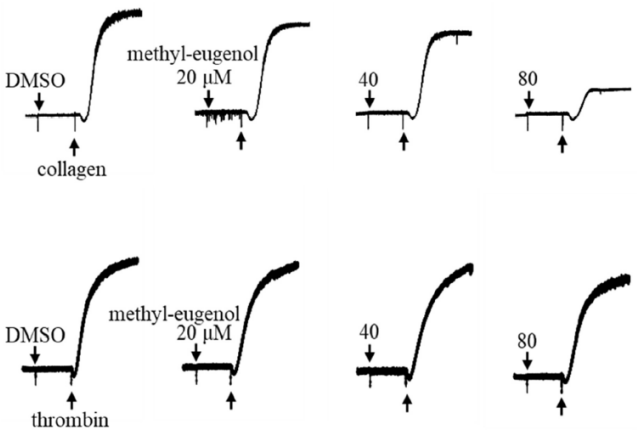

Figure 2: Methyl-eugenol attenuates platelet aggregation induced by collagen or thrombin. Washed human platelets (3.6×10^8 cells/mL) were preincubated with 0.1% DMSO or methyl-eugenol (20–80 µM) prior to stimulation with collagen (2 µg/mL) or thrombin (0.04 U/mL). Data represent four independent experiments.

Methyl-eugenol-mediated regulation of ATP secretion and P-selectin surface expression in platelets

Methyl-eugenol, when administered at concentrations of 40 and 80 µM, exhibited a concentration-dependent reduction in collagen-induced ATP release, as depicted in Figure 3. P-selectin, a pivotal biomarker for platelet activation, typically resides within the inner walls of α-granules. Upon activation, platelets unveil the inner granule contents to the outer membrane [8]. In Figure 4, the suppressive effect of methyl- eugenol on collagen-stimulated surface FITC-P-selectin expression is illustrated (a, Tyrode's solution, 48 ± 14; b, 0.1% DMSO + collagen group, 394 ± 49; c, 40 µM methyl-eugenol + collagen group, 169 ± 16; d, 80 µM methyl-eugenol + collagen group, 122 ± 18; n = 4).

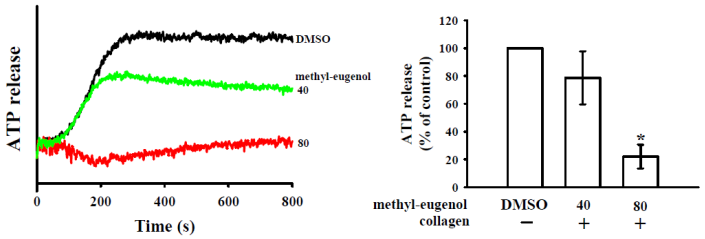

Figure 3: Methyl-eugenol suppresses collagen-induced ATP release in human platelets. Washed platelets (3.6×10^8 cells/mL) were pretreated with 0.1% DMSO or methyleugenol (40 or 80 µM) before stimulation with collagen (2 µg/mL). ATP release (AU; arbitrary unit) and statistical analyses are shown. Data represent mean ± SEM (n = 4). *p < 0.001 vs. 0.1% DMSO control.

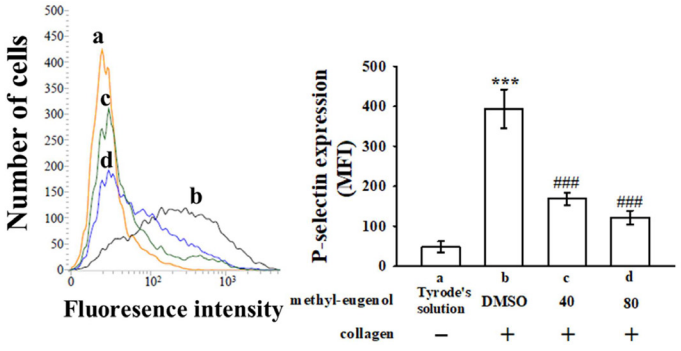

Figure 4: Methyl-eugenol reduces collagen-induced P-selectin expression on human platelets. Washed platelets (3.6×10^8 cells/mL) were treated with 0.1% DMSO or methyl-eugenol (40 or 80 µM) and stimulated with collagen (2 µg/mL). Surface expression of P-selectin was quantified by flow cytometry and expressed as mean fluorescence intensity (MFI): (a) Tyrode's solution; (b) 0.1% DMSO + collagen group; (c) methyl-eugenol 40 µM + collagen group (d) methyl-eugenol 80 µM + collagen group; Values are mean ± SEM (n = 4). ***p < 0.001 vs. Tyrode's solution; ###p < 0.001 vs. 0.1% DMSO control.

Discussion

Methyl-eugenol (Figure 1B), a structural analogue of eugenol, occurs naturally in various plants, including *Croton malambo*, *Cinnamomum cordatum*, and *Melaleuca bracteata* [9]. This compound is commonly found across a wide range of plant species, especially in aromatic spices and traditional medicinal

herbs. Our study demonstrates that methyl-eugenol possesses a notable capacity to inhibit collagen-induced platelet activation, with significant effects observed at concentrations as low as 80 μM. This inhibitory action, however, was not reproduced under thrombin stimulation, highlighting a degree of agonist-specificity in its mechanism of action. Such selectivity suggests that methyl-eugenol may interfere with signaling pathways downstream of collagen receptors, such as glycoprotein VI (GPVI), while leaving thrombin-mediated protease-activated receptor (PAR)-dependent pathways largely intact. This mechanistic distinction is particularly meaningful, as conventional antiplatelet agents such as aspirin or $P2Y_{12}$ antagonists tend to exert broad inhibitory effects, which, while effective, are often accompanied by an elevated risk of hemorrhagic complications [12-13]. By contrast, a compound like methyl-eugenol that selectively attenuates collagen-driven platelet activation could provide therapeutic benefits in conditions where vascular injury and collagen exposure are predominant triggers of thrombosis, such as atherosclerotic plaque rupture. These observations position methyl-eugenol as a potentially valuable adjunct in antithrombotic therapy. Its selective action raises the possibility of synergistic use alongside existing agents, achieving additive protection against arterial thrombosis while minimizing bleeding risk. Furthermore, the identification of natural compounds with such pathway-specific effects expands the pharmacological landscape for antiplatelet drug discovery, offering opportunities to develop agents that refine efficacy-safety balance beyond what is currently achievable with standard therapies.

Platelet activation is a tightly regulated process that initiates with the stimulation of surface receptors and proceeds through a series of intracellular signaling cascades, prominently involving tyrosine kinase pathways. One of the most characteristic outcomes of this signaling network is the exocytotic release of platelet granules, which contain a diverse repertoire of bioactive molecules essential for amplifying platelet activation and stabilizing thrombus formation [4-5]. Among the three principal types of platelet granules—α-granules, dense granules, and lysosomes—α-granules represent the predominant storage compartment. They harbor an extensive array of proteins, including membrane-associated molecules such as P-selectin, as well as soluble factors such as fibrinogen, von Willebrand factor, and platelet-derived growth factor. The mobilization and subsequent release of α-granules not only reinforce platelet-platelet interactions but also contribute to the structural consolidation of the forming thrombus [14-15]. Importantly, the externalization of P-selectin on the platelet surface following α-granule exocytosis is widely regarded as a robust molecular marker of platelet activation, allowing precise assessment of the activation state under experimental and clinical conditions. In this study, the ability of methyl-eugenol to suppress P-selectin expression provides direct evidence of its inhibitory effect on α-granule release. This observation strongly supports the conclusion that methyl-eugenol interferes with one of the central effector mechanisms of platelet activation, thereby attenuating the pro- thrombotic potential of activated platelets.

Conclusion

Collectively, these results underscore the pharmacological promise of methyl-eugenol as a naturally derived antiplatelet agent. By curbing collagen-evoked platelet activation and tempering α-granule release, it exerts a broad inhibitory influence on hemostatic regulation. Moving forward, rigorously designed translational studies—clarifying pharmacokinetics, optimizing structural analogues, and conducting clinical validation—will be crucial to determine whether this compound can progress use to a clinically deployable antithrombotic therapy.

Acknowledgements

This work supported by National Science and Technology Council (NSTC 112-2320- B-038-037-MY3), Taipei Medical University (110-5432-018-111) and Shin Kong Wu Ho-Su Memorial Hospital (2022SKHADR027).

References

1. Lippi G, Franchini M, Targher G. Arterial thrombus formation in cardiovascular disease. Nat Rev Cardiol. 2011; 8: 502-512.
2. Vorchheimer DA, Becker R. Platelets in atherothrombosis. Mayo Clin Proc. 2006; 81: 59-68.
3. Machlus KR, Italiano JE Jr. The incredible journey: From megakaryocyte development to platelet formation. J Cell Biol. 2013; 201: 785-796.
4. Nieswandt B, Watson SP. Platelet-collagen interaction: is GPVI the central receptor?. Blood. 2003; 102: 449-461.
5. Rivera J, Lozano ML, Navarro-Núñez L, et al. Platelet receptors and signaling in the dynamics of thrombus formation. Haematologica. 2009; 94: 700-711.
6. Ulanowska M, Olas B. Biological properties and prospects for the application of eugenol-a review. Int J Mol Sci. 2021; 22: 3671.
7. Marchese A, Barbieri R, Coppo E, et al. Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. Crit Rev Microbiol. 2017; 43: 668-689.
8. Huang WC, Shu LH, Kuo YJ, et al. Eugenol suppresses platelet activation and mitigates pulmonary thromboembolism in humans and murine models. Int J Mol Sci. 2024; 25: 2098.
9. Tan KH, Nishida R. Methyl eugenol: its occurrence, distribution, and role in nature, especially in relation to insect behavior and pollination. J Insect Sci. 2012; 12: 56.
10. Islam MA, Medha MM, Nahar AU, et al. Cancer Protective role of selected dietary polyphenols via modulating Keap1/Nrf2/ARE and interconnected signaling pathways. Nutr Cancer. 2023; 75: 1065-1102.
11. Hsia CW, Shu LH, Lee AW, et al. Ginkgetin effectively mitigates collagen and AA-induced platelet activation via PLCγ2 but not cyclic nucleotide-dependent pathway in human. J Cell Mol Med. 2024; 28: e18139.
12. Stanger L, Yamaguchi A, Holinstat M. Antiplatelet strategies: past, present, and future. J Thromb Haemost. 2023; 21: 3317-3328.

13. Gawaz M, Geisler T, Borst O. Current concepts and novel targets for antiplatelet therapy. Nature Reviews Cardiology. 2023; 20: 583-599.

14. Blair P, Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. Blood Rev. 2009; 23: 177-189.

15. Heijnen H, van der Sluijs P. Platelet secretory behaviour: as diverse as the granules … or not?. J Thromb Haemost. 2015; 13: 2141-2151.