Research Article

www.ijrap.net

(ISSN Online:2229–3566, ISSN Print:2277–4343)

SYNERGISTIC EFFECT OF *ZINGIBER OFFICINALE* AND *ALLIUM SATIVUM* AGAINST PLATELET AGGREGATION

Mohamed Fiaz A [1]*, Anand Babu K [2]

[1] Associate Professor, Department of Pharmacology, Sree Abirami College of Pharmacy, Coimbatore, Tamilnadu, India

[2] Professor, Department of Pharmaceutical Analysis, GRT Institute of Pharmaceutical Education & Research, Tiruttani, Tamilnadu, India

Received on: 11/12/25 Accepted on: 12/1/26

***Corresponding author**
E-mail: fiaz0306raheem@gmail.com

DOI: 10.7897/2277-4343.17116

ABSTRACT

Platelet aggregation, a pathological process in which platelets adhere to blood vessels and release inflammatory mediators, contributes to vascular and cardiac complications. This study evaluated the in-vitro synergistic anti-platelet activity of *Zingiber officinale* (ginger) and *Allium sativum* (garlic). Fresh and dried extracts were prepared using the maceration technique, and phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, and glycosides. The anti-platelet effect was assessed using platelet-rich plasma and platelet-poor plasma obtained from a healthy poultry bird. A mixture of garlic and ginger extracts in a 5:1 ratio demonstrated significant inhibition of platelet aggregation in both fresh ($p < 0.01$) and dried ($p < 0.01$) extracts, with dried extracts exhibiting the highest percentage of platelet inhibition. These findings suggest that the combination of *Zingiber officinale* and *Allium sativum* has potent synergistic anti-platelet activity, supporting its potential role as a natural therapeutic agent against platelet-mediated vascular disorders.

Keywords: *Zingiber officinale, Allium sativum*, platelet aggregation, anti-platelet activity, phytochemicals

INTRODUCTION

Medicinal plants have been used in healthcare for centuries, playing a crucial role in disease prevention and treatment[1]. Their natural compounds provide medicinal properties, making them useful for curing various diseases and generating income. Ayurveda and other Indian literature mention their use in treating various ailments[1]. This study focuses on understanding the medicinal uses of plants and scientific investigation to confirm their medicinal values. Thrombosis is mainly driven by platelet activation and aggregation[2]. With the rising incidence of thrombotic disorders, there is an increasing need for novel anti-platelet agents that can effectively prevent and manage arterial thrombosis while reducing unwanted side effects[3-5]. Traditional medicinal herbs have been used for centuries to treat various human diseases, and many safe and effective compounds have been identified through pharmacological investigations[6-8]. As complementary therapeutic options, traditional medicinal herbs offer significant potential for drug development. However, their clinical use is often constrained by limited understanding of their efficacy and biological mechanisms[9]. Consequently, detailed studies on the mechanisms of action of traditional medicinal herbs are essential to support their use as alternative therapeutic agents[10]. This study highlights current insights into the mechanisms by which traditional medicinal herbs regulate platelet function and exert anti-platelet and antithrombotic effects[11].

Anti-platelet activity in Ginger

Gingerol compounds and their derivatives are more potent anti-platelet agents than aspirin[12]. [8]-Paradol, a natural constituent of ginger, was found to be the most potent COX-1 inhibitor and anti-platelet aggregation agent[13]. The mechanism underlying AA-induced platelet aggregation inhibition may be related to attenuation of COX-1/Tx synthase enzymatic activity[14].

Anti-platelet activity in Garlic

Garlic exhibits cholesterol- and lipid-lowering, antihypertensive, and antioxidant properties, making it effective in the prevention and treatment of cardiovascular diseases[15]. It also possesses antithrombotic, fibrinolytic, and antiplatelet activities. Alliin, the major sulfur-containing compound in garlic, is converted to allicin by the enzyme alliinase when garlic is crushed or chewed[16]. Allicin is considered the primary bioactive component responsible for garlic's pharmacological effects. Additionally, ajoene, a secondary metabolite of alliin, has potent antiplatelet activity and has been shown to synergistically enhance the effects of antiplatelet drugs such as forskolin, indomethacin, and dipyridamole[17,18].

MATERIALS AND METHODS

Collection of sample

Ginger (*Zingiber officinale*) rhizomes and garlic (*Allium sativum*) bulb were purchased from local vegetable market in Anjarakandy, Kannur, Kerala, India.

Sample Preparation

Sample 1 (Dried sample)

The Ginger (*Zingiber officinale*) was peeled and cut into small pieces then it was dried in sunlight for 1 week and dried ginger was collected for extraction process[18]. The skin of Garlic (*Allium sativum*) was peeled off and was cut into small pieces, then it was dried in sunlight for 10 days followed by drying in shade for 2 days. The dried garlic and ginger were then subjected as a mixture in 5:1 ratio to extraction process[19,20].

Sample 2 (Fresh sample)
For conducting fresh studies, the Ginger and Garlic (5:1) was used as a mixture for extraction process using alcohol (99.5% methanol).

Extraction of sample
Extraction of dried samples: The dried garlic and ginger was grinded separately in a mixer grinder until fine powder of each is obtained. 5g of dried ginger powder and 1g of garlic powder were weighed using sensitive balance and then suspended in 100 ml of methanol in conical flask with continuous shaking by extractive exhaustion method till maximum extraction is achieved[21,22]. Strain the solution using filter paper no. 4 and then the filtrate was collected in a container the filtrate was dried in an room temperature at 25 ^{0}C and the final dried product was stored in freezer at -10 ^{0}C throughout the study[23].

Extraction of Fresh sample: Fresh garlic and ginger was peeled off and cut into small pieces and were grinded in a mixer grinder separately till maximum grinding was achieved. The grinded ginger and garlic were weighed using sensitive balance in ratio of 5:1 and then suspended in 100 ml of methanol in conical flask with continuous shaking for 24 hours (maceration)[24]. Then it was allowed to extract for 3 days in methanol solution. Strain the solution using filter paper no.4 and then the filtrate was collected in a container[25]. The filtrate was dried in oven at 100oc for 30 min, Then the final dried product was stored in freezer at -10 ^{0}C throughout study[26,27].

Phytochemical Screening

Table 1: Phytochemical screening of mixture of dried garlic and ginger

Test	Procedure	Inference
Mayers test	1ml of filtrate add Dil. HCl, shaken well and filter. Take a small amount of acidic extract and add 2- drops of Mayer's reagent down the side	Creamy precipitate seen Presence of Alkaloid
Molisch test	2ml of sample add 2- drops of Molisch's reagent. Tilt the test tube add 1-2 ml of concentrated sulfuric acid	Violet ring forms at junction Presence of Carbohydrate
Alkaline reagent test	To a 2ml of sample extract add 2-3 drops of NaOH solution. An intense yellow color forms. Add a few drops of dilute HCl to the yellow solution.	Intense yellow color formation with NaOH followed by disappearance of color upon adding dil HCl Presence of Flavonoids
Bromine water test	Add the test solution to the 2ml of bromine water	Yellow colour precipitate seen Presence of Glycoside

Table 2: Phytochemical screening of fresh mixture of Ginger and garlic

Test	Procedure	Inference
Mayers test	1ml of filtrate add Dil. HCl, shaken well and filter. Take a small amount of acidic extract and add 2- drops of Mayer's reagent down the side	Creamy precipitate seen Presence of Alkaloid
Molisch test	2ml of sample add 2- drops of Molisch's reagent. Tilt the test tube add 1-2 ml of concentrated sulfuric acid	Violet ring forms at junction Presence of Carbohydrate
Alkaline reagent test	To a 2ml of sample extract add 2-3 drops of NaOH solution. An intense yellow color forms. Add a few drops of dilute HCl to the yellow solution.	Intense yellow color formation with NaOH followed by disappearance of color upon adding dil HCl Presence of Flavonoids
Bromine water test	Add the test solution to the 2ml of bromine water	Yellow colour precipitate seen Presence of Glycoside

***In-vitro* Anti-Platelet Activity**
To prepare platelet-poor plasma (PPP) and platelet-rich plasma (PRP), whole blood was collected from a poultry bird and stored in an anti-coagulant tube such as EDTA, citrate, or heparin, ensuring thorough mixing by gently inverting the tubes. Subsequently, a clean centrifuge tube was labelled as "PPP," and the whole blood was carefully transferred into it using a sterile pipette, taking care to avoid disturbing the sedimented cells at the bottom of the anticoagulant tube. The whole blood underwent centrifugation at a relatively high speed (e.g., 1500-2000 x g) for 10-15 minutes at room temperature. After centrifugation, three distinct layers were visible in the tube: plasma at the top, the buffy coat (comprising white blood cells and platelets) in the middle, and red blood cells at the bottom[28].

Plasma was then meticulously collected from the upper layer using a pipette, ensuring the buffy coat and red blood cell layer remained undisturbed. This plasma was transferred to a clean centrifuge tube labelled as "PPP" and centrifuged again at a higher speed (e.g., 3000-5000 x g) for an additional 10-15 minutes to completely remove platelets[29].

The resulting platelet-poor plasma (PPP) was collected from the top layer and transferred to a clean container for further use or storage. In parallel, platelet-rich plasma (PRP) was prepared by following similar steps as for PPP but centrifuging the whole blood at a relatively low speed (e.g., 150-250 x g) for 10-15 minutes.

PRP was carefully collected from the layer just above the buffy coat and transferred to a clean centrifuge tube labelled as "PRP." Adjustments to the platelet concentration in PRP could be made, if necessary, through dilution or concentration using the centrifuge. Finally, the PRP was ready for utilization in various experiments or clinical procedures. Proper aseptic techniques were employed throughout to prevent contamination, and centrifugation conditions were adjusted as needed for optimal results[30,31].

Anti-platelet Activity Assay: A 100µL PRP and PPP, was made up to 150 µL using distilled water. The absorbance of PRP was measured using a spectrophotometer at a wavelength of 600nm, with PPP serving as the blank. This is marked as OD [T1] Treatment and incubation: To each test tube containing a 100µL PRP or 100µL PPP, 50µL of 100 mg/ml test samples was added, and the mixture was incubated at 37°C for 20 minutes. Platelet aggregation was initiated with induction by arachidonic acid (0.5mM). The absorbance of PRP was measured using a spectrophotometer at a wavelength of 600nm, with PPP serving

as the blank[32]. This is marked as OD [T2]. Control groups were maintained without the addition of samples.

Their absorbance before and after incubation were marked as OD[T3] and OD[T4] respectively.

Platelet aggregation % in test samples = $([T1]-OD[T2])OD[T1\} \times 100$
Platelet aggregation % in control samples = $([T3]-OD[T4])OD[T4\} \times 100$

Aggregation inhibition % = (Platelet aggregation in control−Platelet aggregation in test) Platelet aggregation in control × 100

Figure 1: Rhizomes of Ginger

Figure 2: Garlic bulb

Figure 3: Extract of dried garlic and ginger mixture

Figure 4: Extract of fresh garlic and ginger mixture

RESULT

Table 3: Percentage Platelet inhibition of Garlic and Ginger extract alone

Sample	% Platelet Inhibition
Garlic (1µg/µL)	10.371±0.23
Ginger (5µg/µL)	18.375±1.32

Table 4: Percentage of platelet inhibition in Mixture of Garlic and Ginger extract

Sample	OD		% Platelet Aggregation	% Platelet Inhibition
Fresh[S1] Ginger and Garlic Ratio:(5:1) Concentration(50µL)	**[T1**	0.2759	32.08	
	[T2]	0.1874		
	[T3]	0.2698	46.81	31.48±0.12**
	[T4]	0.1435		
Dry[S2] Ginger and Garlic Ratio:(5:1) Concentration(50µL)	**[T1]**	0.2513	28.85	
	[T2]	0.1688		
	[T3]	0.2676	4.09	33.04±0.22**
	[T4]	0.1523		
Standard (Aspirin) Concentration(100µg/ml)	**[T1]**	0.2634	38.06	
	[T2]	0.1744		
	[T3]	0.2746	48.45	42.45±0.14***
	[T4]	0.1545		

Values are expressed as mean ± SEM, p<0.05*,p<0.01**,p<0.001*** as compared with garlic or ginger extract alone

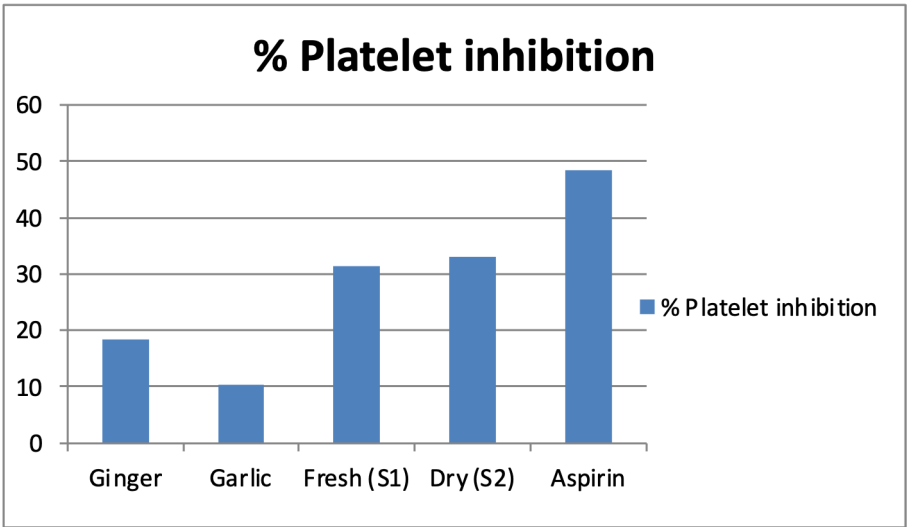

Figure 5: % Platelet inhibition of samples against Garlic and Ginger

DISCUSSION

Platelet hyperactivity is a major contributor to thrombotic disorders, and natural agents with anti-platelet properties are of growing interest due to their safety profile. While *Zingiber officinale* (ginger) and *Allium sativum* (garlic) have individually demonstrated modest antiplatelet effects, their combined potential had remained unexplored. In this study, we demonstrate that the combination of ginger and garlic extracts produces significantly greater inhibition of platelet aggregation compared with either agent alone **(**$p < 0.01$**),** indicating a synergistic interaction. This enhanced activity likely arises from complementary bioactive compounds**—**gingerols and shogaols in ginger, and allicin and ajoene in garlic—which converge to inhibit thromboxane A_2 synthesis and downstream platelet activation. Notably, dried alcoholic extracts exhibited slightly higher potency than fresh extracts, suggesting that drying may concentrate or convert bioactive constituents into more efficacious forms, thereby enhancing their antiplatelet effect[25].

Our findings exemplify the concept of polypharmacology**,** where multi-component botanical combinations target overlapping pathways to produce greater therapeutic outcomes. The observed synergy highlights the potential of ginger–garlic combinations as natural adjuncts for thrombotic risk management**,** particularly in populations with contraindications to conventional anti-platelet therapy.

While these *in vitro* results are promising, further *in vivo* and clinical studies are warranted to evaluate pharmacokinetics, bioavailability, and therapeutic efficacy in humans. Collectively, this study provides a mechanistic and translational rationale for developing standardized herbal formulations combining ginger and garlic as a novel, multi-targeted anti-platelet strategy.

CONCLUSION

The study evaluated the antiplatelet activity of combined *Zingiber officinale* and *Allium sativum* using two preparations: fresh (S1) and dried (S2) extracts. Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared from blood collected in sodium citrate tubes, treated with the test samples, and incubated. Platelet aggregation was then induced using arachidonic acid, and absorbance was measured before and after treatment. The results demonstrated that the combination of garlic and ginger produced a synergistic anti-platelet effect compared with either agent alone. Both fresh and dried extracts inhibited platelet aggregation, with the dried extract showing a higher percentage of inhibition than the fresh extract.

ACKNOWLEDGEMENT

The present work was supported by College of Pharmacy, Kannur Medical College, Anjarakandy as an academic project for the Degree of Bachelor of Pharmacy. The authors are thankful to the management team and Principal, College of Pharmacy, Kannur.

REFERENCES

1. Eikelboom JW, Hirsh J, Spencer FA, Baglin TP, Weitz JI. Antiplatelet drugs. Chest. 2012;141(2 Suppl):e89S–e119S.
2. MedlinePlus. Atherosclerosis [Internet]. Blood, Heart and Circulation; 2010 [cited 2024 May 14]. Available from: https://medlineplus.gov/atherosclerosis.html
3. Cleveland Clinic. Thrombocytopenia [Internet]. [cited 2024 May 14]. Available from: https://my.clevelandclinic.org/health/diseases/14430-thrombocytopenia
4. Mayo Clinic. Coronary artery disease [Internet]. 2022 [cited 2024 May 14]. Available from: https://www.mayoclinic.org/diseases-conditions/coronary-artery-disease/symptoms-causes/syc-20350613
5. Iqbal AM, Lopez RA, Hai O. Antiplatelet medications [Internet]. StatPearls; 2025 Jan [updated 2022 Nov 7; cited 2024 May 15]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537062/
6. Sarafoff N, Byrne RA, Sibbing D. Clinical use of clopidogrel. Curr Pharm Des [Internet]. 2012 [cited 2024 May 15];18(33):5224–39. Available from: https://www.eurekaselect.com/article/45958
7. Garg AK. Role of medicinal plants in human health diseases. Asian J Plant Sci Res. 2021;11(1):19–21.
8. Nurtjahja-Tjendraputra E, Ammit AJ, Roufogalis BD, Tran VH, Duke CC. Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. Thromb Res. 2003;111(4–5):259–65.
9. Shafiekhani M, Faridi P, Kojuri J, Namazi S. Comparison of antiplatelet activity of garlic tablets with cardio-protective dose of aspirin in healthy volunteers: a randomized clinical trial. Avicenna J Phytomed. 2016;6(5):550–6.
10. Mošovská S, Nováková D, Kaliňák M. Antioxidant activity of ginger extract and identification of its active components. Acta Chim Slovaca [Internet]. 2015;8(2):115–20. Available from: http://dx.doi.org/10.1515/acs-2015-0020
11. Karim A, Rasul A. Development, characterization and evaluation of *in-vitro* anti-inflammatory activity of ginger extract-based microemulsion. 2019;32(3):1327–32.
12. Islam K, Rowsni AA, Khan MM, Kabir MS. Antimicrobial activity of ginger (*Zingiber officinale*) extracts against food-borne pathogenic bacteria. Int J Sci Environ Technol. 2014;3(3):867–71.
13. Ansari JA, *et al*. Anticancer and antioxidant activity of *Zingiber officinale* Roscoe rhizome. Indian J Exp Biol. 2016;54(11):767–73.
14. Shanmugam KR, Mallikarjuna K, Kesireddy N, Reddy KS. Neuroprotective effect of ginger on antioxidant enzymes in streptozotocin-induced diabetic rats. Food Chem Toxicol. 2011;49(4):893–7.
15. Taha NM, Mandour AA. Antioxidative and cardioprotective effects of ethanolic extract of ginger on Triton WR-1339-induced hyperlipidemia in rats. J Nat Sci Res. 2014;6(1):153–8.
16. Chang JS, Wang KC, Yeh CF, Shieh DE, Chiang LC. Fresh ginger (*Zingiber officinale*) has antiviral activity against human respiratory syncytial virus. J Ethnopharmacol. 2013;145(1):146–51.
17. Ebrahimzadeh Attari V, Malek Mahdavi A, Javadivala Z, Mahluji S, Zununi Vahed S, Ostadrahimi A. Anti-obesity and weight-lowering effects of ginger: a systematic review. Phytother Res. 2018;32(4):577–85.
18. Palatty PL, Haniadka R, Valder B, Arora R, Baliga MS. Ginger in the prevention of nausea and vomiting: a review. Crit Rev Food Sci Nutr. 2013;53(7):659–69.
19. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. London: Chapman and Hall; 1998.
20. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 55th ed. Pune: Nirali Prakashan; 2019.
21. Trease GE, Evans WC. Trease and Evans pharmacognosy. 16th ed. London: Saunders Elsevier; 2009.
22. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4(3):196.
23. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of

bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8(1):1–10.
24. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, *et al*. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal. 2014;22(3):296–302.
25. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Int Pharm Sci. 2011;1(1):98–106.
26. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules. 2009;14(6):2167–80.
27. Hussein J, Hussein H, *et al.* A review: antimicrobial, anti-inflammatory and cardiovascular effects of garlic [Internet]. Res J Pharm Technol; [cited 2024 May 14]. Available from: www.rjptonline.org
28. Thomson M, Ali M. Garlic (*Allium sativum*): a review of its potential use as an anti-cancer agent. Curr Cancer Drug Targets. 2003;3(1):67–81.
29. Born GVR. Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature. 1962;194:927–9.
30. Nurtjahja Tjendraputra E, Ammit AJ, Roufogalis BD, Tran VH, Duke CC. Effective anti-platelet and COX-1 enzyme inhibitors from pungent constituents of ginger. Thromb Res. 2003;111(4–5):259–65.
31. Bordia T, Mohammed N, Thomson M, Ali M. Evaluation of garlic and onion as antithrombotic agents. Prostaglandins Leukot Essent Fatty Acids. 1996;54(3):183–6.
32. Shafiekhani M, Faridi P, Kojuri J, Namazi S. Comparison of antiplatelet activity of garlic tablets with cardio-protective dose of aspirin in healthy volunteers. Avicenna J Phytomed. 2016;6(5):550–6.
33. Michelson AD. Platelets. 3rd ed. London: Academic Press; 2013.
34. Al-Qattan KK, Alnaqeeb MA, Ali M. Antihypertensive effect of garlic (*Allium sativum*) in the two-kidney one-clip Goldblatt model. J Ethnopharmacol. 1999;66(2):217–22.
35. Strika I, *et al*. Antimicrobial effects of garlic (*Allium sativum* L.). Bull Chem Technol Bosnia Herzegovina. 2016;47:17–20.
36. Sripanidkulchai B. Benefits of aged garlic extract on Alzheimer's disease: possible mechanisms of action. Exp Ther Med [Internet]. 2020;19(2):1560–1564.
37. Bordia T, Mohammed N, Thomson M, Ali M. Evaluation of garlic and onion as antithrombotic agents. Prostaglandins Leukot Essent Fatty Acids [Internet]. 1996;54(3):183–186.
38. Sutarno H, Hadad EA, Brink M. *Zingiber officinale* Roscoe. In: De Guzman CC, Siemonsma JS, editors. Plant Resources of South-East Asia: No.13: Spices. Backhuys Publishers; 1999. p. 238–44.
39. Wikipedia contributors. Garlic [Internet]. Wikipedia, The Free Encyclopedia; [cited 2024 May 14]. Available from: https://en.m.wikipedia.org/wiki/Garlic
40. Augustina L, Gan E. *In-vitro* antiplatelet activities of aqueous extract of garlic. Res J Pharm Technol. 2022;15(4):1579–1582.

Cite this article as:

Mohamed Fiaz A and Anand Babu K. Synergistic effect of *Zingiber officinale* and *Allium sativum* against Platelet aggregation. Int. J. Res. Ayurveda Pharm. 2026;17(1):92-96
DOI: http://dx.doi.org/10.7897/2277-4343.17116

Source of support: College of Pharmacy, Kannur Medical College, Anjarakandy, Kannur, Kerala, India, Conflict of interest: None Declared