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Multitarget Antithrombotic Effects of Scutellarin On Platelet–Coagulation Hemostasis

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Abstract: Background: Scutellarin, a flavonoid-rich compound derived from Erigeron breviscapus–related plant material, has been reported to exert cardiovascular and antithrombotic effects, but its integrated influence on platelet–coagulation hemostasis remains incompletely characterized. Methods: we analyzed the in vitro effects of scutellarin on thrombin time (TT), activated recalcification time (ART), activated partial thromboplastin time (APTT), prothrombin time (PT), and ADP-induced platelet aggregation, as well as its effects on intracellular Ca2+ signaling in platelets. Results: Scutellarin prolonged TT, ART, APTT, and PT in a concentration-dependent manner, indicating a broad anticoagulant profile spanning both intrinsic and extrinsic/common coagulation pathways. TT increased from a baseline range of 18–25 s to as high as 140 s at 50 μM, while PT reached approximately 85 s at the upper tested concentrations. In platelet studies, scutellarin strongly inhibited the second phase of ADP-induced aggregation and attenuated ADP-evoked intracellular Ca2+ rise by approximately 16–19% without behaving as a simple extracellular Ca2+ chelator. Platelet counts were not significantly reduced. Conclusions: The available experimental data support scutellarin as a multitarget modulator of platelet–coagulation hemostasis with both antiplatelet and anticoagulant properties. The pattern of response is consistent with interference at several levels, including thrombin/fibrin formation, coagulation-complex assembly on phosphatidylserine-rich membranes, and receptor-linked platelet Ca2+ signaling.

Keywords: Scutellarin; platelet aggregation; coagulation; thrombin time; activated partial thromboplastin time;

prothrombin time; intracellular calcium; flavonoid; antithrombotic agent.

Introduction: Thrombosis is a multicomponent process arising from the dynamic interaction of vascular wall injury or dysfunction, platelets, plasma coagulation factors, blood flow, and inflammatory signaling [1,2]. Although arterial thrombi are usually platelet-rich and venous thrombi are relatively fibrin- and erythrocyte-rich, platelet activation and thrombin generation are functionally linked in both settings. Thrombin is not only the central enzyme of fibrin formation but also a potent platelet agonist, whereas activated platelets externalize phosphatidylserine (PS) and thereby provide a negatively charged catalytic platform for the intrinsic tenase and prothrombinase complexes [3–7].

From the perspective of cell-based hemostasis, the initiation phase begins on tissue factor-bearing surfaces, whereas amplification and propagation occur predominantly on activated platelet membranes [1,7]. This framework helps explain why integrated anticoagulant phenotypes may be observed even when a test appears to probe only one nominal pathway. Standard clotting assays such as PT, APTT, and TT remain useful because they identify pathway-sensitive shifts in fibrin formation, whereas thrombin generation and thromboelastography provide a more global picture of coagulation kinetics and clot quality [8–11].

Flavonoids and related plant-derived polyphenols have attracted attention as candidate multitarget antithrombotic agents. Several flavonoids can inhibit coagulation factor Xa, attenuate thrombin amidolytic activity, reduce platelet aggregation, and modulate intracellular signaling pathways involved in Ca2+ mobilization and granule release [12–18]. Scutellarin, a principal flavonoid associated with breviscapine preparations, has been linked to vasodilatory, endothelial-protective, anti-inflammatory, antithrombotic, and anticoagulant actions in cardiovascular research [17]. However, the mechanistic profile of scutellarin across both platelet and coagulation compartments has not been fully defined under assay conditions that combine clotting endpoints, viscoelastic testing, and platelet Ca2+ signaling.

The working hypothesis was that scutellarin exerts a broad hypocoagulant phenotype in vitro and in vivo while also suppressing platelet activation, especially ADP-dependent secondary amplification, through mechanisms involving impaired coagulation-complex assembly and attenuation of intracellular Ca2+ signaling.

METHODS

Scutellarin was obtained from the Institute of the Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan. Heparin was used as the reference anticoagulant in comparative experiments. Where assay-specific stock formulations differed, concentrations and doses are reported in the units used in the original experiments so as not to distort the underlying data.

Healthy rat plasma served as the main matrix for the in vitro clotting assays. Thrombin time (TT), activated recalcification time (ART), activated partial thromboplastin time (APTT), and prothrombin time (PT) were evaluated after incubation with scutellarin across the concentration ranges reported in the underlying experiments. TT experiments additionally included conditions in which scutellarin was preincubated with fibrinogen or tested in plasma-based systems. APTT was used as a pathway-sensitive index of intrinsic/common pathway activity, PT as an index of extrinsic/common pathway activity, TT as an index of thrombin-mediated fibrin formation, and ART as a global calcium- and phospholipid-dependent clotting measure [1,8,11].

Platelet function was assessed by ADP-induced aggregation experiments. The source data indicated that scutellarin altered the first phase of aggregation and nearly abolished the second phase under selected concentrations. Additional experiments examined the modifying influence of extracellular Ca2+ over the range of 0.05–1.5 mM. The available mechanistic interpretation was complemented by fluorescence-based measurement of intracellular free Ca2+ using Fura-2/AM in resting and ADP-stimulated platelets.

The underlying figures report most in vitro data as mean values with significance markers and n = 6. Where exact numerical values were reported in the source chapter, they are reproduced in the Results section and in the tables below. Statistical significance in the original experiments was indicated at $p < 0.05$, $p < 0.01$, or $p < 0.001$ relative to control.

RESULTS

1. Scutellarin prolonged thrombin time in vitro μ

In the thrombin time series, scutellarin displayed a prominent anticoagulant phenotype at the terminal stage of coagulation. Thrombin time primarily reflects the conversion of fibrinogen to fibrin after the addition of exogenous thrombin; therefore, TT prolongation usually indicates interference with thrombin catalytic activity, thrombin–fibrinogen interaction, or fibrin polymerization rather than an upstream defect in

contact activation alone. In the presented data set, pre-incubation of thrombin with fibrinogen at a concentration of 30 mg/mL did not result in a clear increase in clotting time, whereas systems containing plasma, thrombin, Ca2+ and fibrinogen showed a marked increase, reaching approximately 140 s for scutellarin at a concentration of 50 µM compared to the baseline range of 28 s (Fig. 1). This pattern argues against gross fibrinogen destabilization as the sole mechanism and is more consistent with an effect at the level of thrombin-mediated fibrin formation or thrombin-dependent catalytic amplification. Related plasma assays have shown that the scutellarin scaffold can prolong TT together with APTT and PT, supporting the interpretation that this flavonoid family has measurable anticoagulant activity in clotting-based assays.

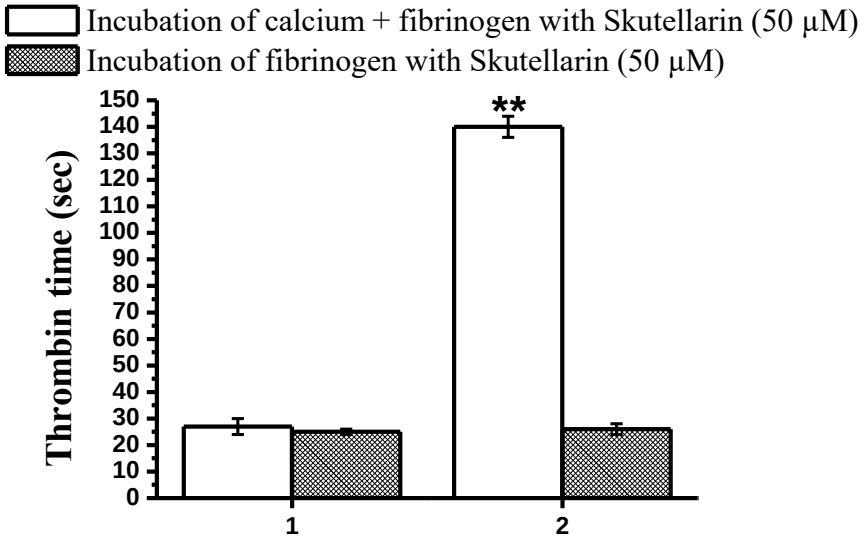

Figure 1. Effect of Skutellarin on thrombin time. 1 - Control; 2 - Skutellarin (50 µM). Reliability index: - P <0.01; (n = 6).**

Mechanistically, a TT-dominant effect can be interpreted in at least three non-exclusive ways. First, scutellarin may reduce thrombin efficiency toward fibrinogen, either by interacting with catalytic or exosite-dependent substrate recognition surfaces. Second, it may alter the physicochemical transition from soluble fibrin monomer to polymerized fibrin network. Third, it may weaken the positive-feedback environment in plasma that normally supports stable terminal clot formation. Because many flavonoids can inhibit thrombin and/or factor Xa as a class effect, and because scutellarin-related compounds have repeatedly shown clotting-time prolongation in thrombin-oriented assays, the current results are compatible with a direct or quasi-direct influence on the terminal thrombin–fibrin axis, although enzyme-kinetic confirmation would still be required.

2. Scutellarin prolonged activated recalcification time

Scutellarin also prolonged activated recalcification time across the tested concentration range of 5–100 µM, indicating a broader hypocoagulant shift than would be expected from isolated inhibition of a single terminal reaction (Fig. 2). Recalcification assays are highly dependent on restoration of ionized Ca^{2+} and on efficient assembly of membrane-bound coagulation complexes after citrate chelation is reversed. For this reason, prolongation of recalcification time is commonly interpreted as evidence of impaired phospholipid-dependent coagulation efficiency, reduced tenase/prothrombinase assembly, or diminished overall thrombin-forming capacity. In mechanistic terms, the result suggests that scutellarin affects not only the final fibrin-forming step but also the upstream organization of coagulation reactions on biologic surfaces.

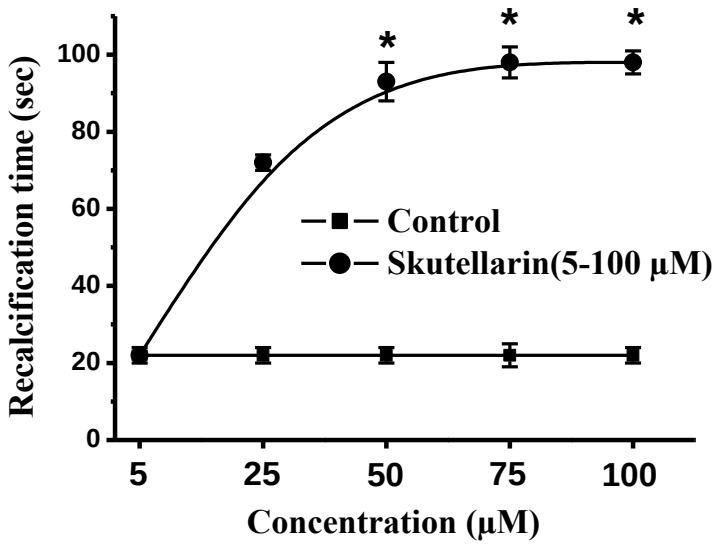

Figure 2. Effect of Scutellarin on activated plasma recalcification time. Reliability index: * - P <0,05. (n = 6).

From a molecular-biophysical perspective, the most plausible explanation is interference with one or more Ca^{2+}-dependent membrane events. Assembly of intrinsic tenase and prothrombinase requires negatively charged phosphatidylserine-rich surfaces and Ca^{2+}-mediated docking of vitamin K-dependent coagulation factors. A compound that reduces procoagulant surface expression, alters membrane organization, dampens platelet-driven phosphatidylserine exposure, or weakens the efficiency of factor anchoring would be expected to prolong recalcification assays. Therefore, the observed phenotype is mechanistically compatible with suppression of coagulation-complex assembly rather than with a purely receptor-independent bulk effect in plasma.

3. Scutellarin prolonged APTT and PT

The concomitant prolongation of APTT and PT indicates that scutellarin does not behave as a narrow pathway-selective inhibitor. APTT mainly captures the intrinsic/common axis, whereas PT is more sensitive to the extrinsic/common pathway. In the reported dataset, APTT was prolonged over 5–50 µM (Fig. 3) and PT increased in a concentration-dependent fashion over 5–100 µM, reaching approximately 85 s at the upper range (Fig. 4). When these findings are considered together with the marked TT effect, the most parsimonious interpretation is that scutellarin slows coagulation kinetics at multiple levels, spanning intrinsic amplification, extrinsic/common-pathway propagation, and terminal thrombin-driven fibrin formation.

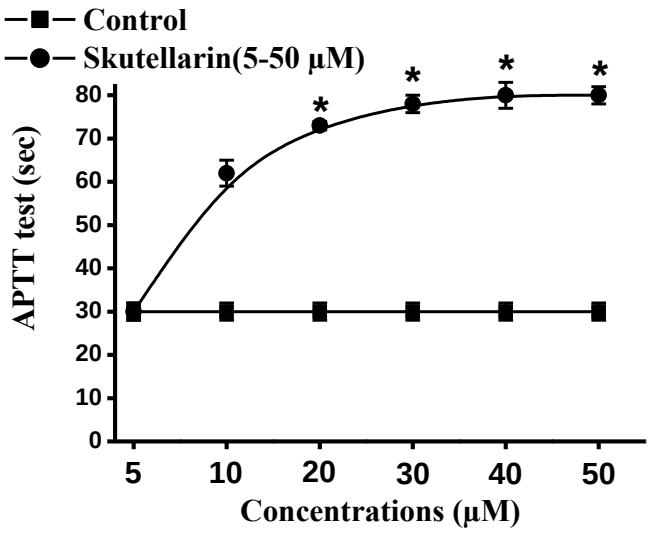

Figure 3. Study of the effect of Scutellarin (5-50 µM) on the APTT test. Reliability index: * - P <0,05. (n = 6).

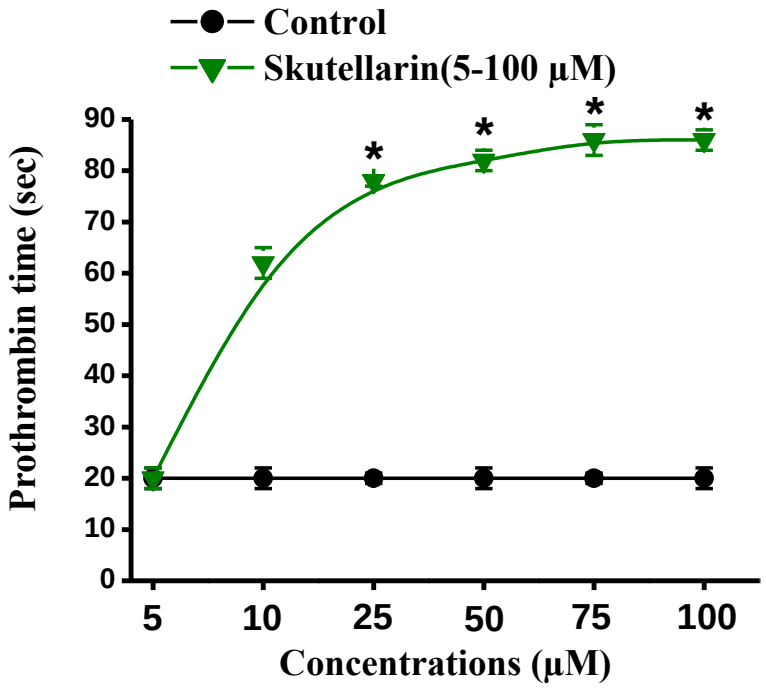

Figure 4. Study of the effect of Scutellarin (5-100 µM) on prothrombin time. Reliability index: * - P <0,05. (n = 6).

Importantly, simultaneous PT/APTT prolongation does not by itself identify a unique molecular target. Rather, it points to a global reduction in clotting efficiency that may arise from combined effects on factor activation, cofactor function, membrane-dependent complex formation, and terminal thrombin output. This interpretation is also supported by prior pharmacological work on scutellarin/scutellarein-related compounds, in which prolongation of TT, APTT, and PT was observed in parallel. Thus, the present coagulation profile is more consistent with broad anticoagulant modulation than with inhibition of only one classical branch of the cascade.

4. Scutellarin inhibited ADP-induced platelet aggregation

Scutellarin strongly inhibited ADP-induced platelet aggregation and, according to the reported dataset, substantially attenuated the first phase while nearly abolishing the second phase at the reported concentration of 10 µg/µL. This distinction is mechanistically important (Fig. 5). ADP-induced platelet activation depends on coordinated signaling through $P2Y_1$ and $P2Y_{12}$ receptors: the $P2Y_1$ arm contributes to shape change and early Ca^{2+} mobilization, while the $P2Y_{12}$ arm is essential for sustained integrin $\alpha IIb\beta_3$ activation, amplification, secretion, and stable aggregate formation. Accordingly, marked suppression of the second phase implies disruption of one or more amplification processes downstream of receptor activation, including dense-granule reinforcement, sustained $\alpha IIb\beta_3$ activation, secondary mediator support, and Ca^{2+}-dependent signaling persistence.

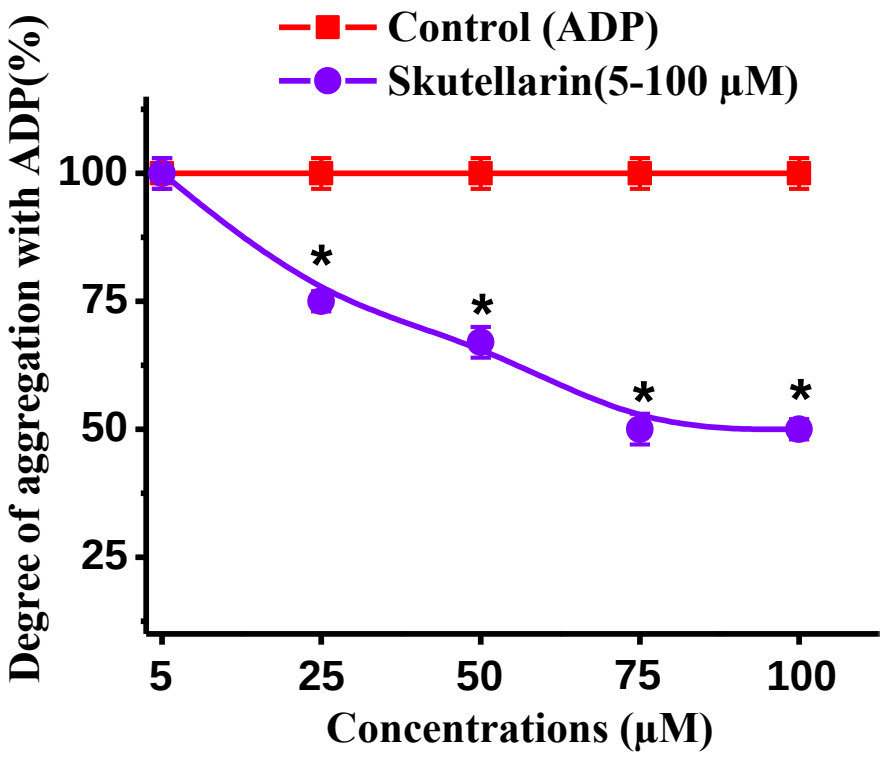

Figure 5. Dose-dependent effect of Scutellarin on spontaneous platelet aggregation induced by ADP. Note: Abscissa axis – platelet aggregation as a percentage; ordinate axis – compound concentration. Reliability index: * - P <0,05. (n = 6).

The observed strengthening of the inhibitory effect in the presence of extracellular Ca^{2+} further supports a mechanism beyond passive steric inhibition. In platelets, extracellular Ca^{2+} does not simply serve as an inert ion reservoir; it also influences nucleotide metabolism, integrin-dependent aggregation, and the stability of ADP-driven responses. Experimental work has shown that extracellular Ca^{2+} modulates ADP-evoked aggregation through ectonucleotidase-dependent agonist degradation and through effects on secondary amplification. Therefore, the Ca^{2+}-dependence of scutellarin's anti-aggregatory effect is compatible with interference in signaling systems that require sustained ADP responsiveness rather than with a purely surface-blocking mechanism.

A further mechanistic clue comes from the closely related metabolite scutellarein. In rat washed platelets, scutellarein inhibited adhesion and aggregation induced by thrombin, U46619, and ADP, while exerting only a mild effect on intracellular Ca^{2+} mobilization; in that study, its intracellular target profile was linked primarily to protein kinase C (PKC), rather than to simple receptor antagonism or calcium scavenging. Because scutellarein is a principal in vivo metabolite of scutellarin and shares the same flavone core, the current scutellarin phenotype is plausibly interpreted within the same signaling space—namely, suppression of platelet amplification pathways downstream of surface receptor engagement.

5. Scutellarin attenuated ADP-induced platelet intracellular Ca^{2+} rise without acting as a simple calcium chelator

In intact platelets, scutellarin did not reproduce the behavior of EGTA and therefore cannot be interpreted as a simple extracellular Ca^{2+} chelator. This distinction is mechanistically critical. EGTA reduces the extracellular Ca^{2+} pool directly and therefore strongly suppresses the external Ca^{2+}-dependent component of the platelet response. By contrast, scutellarin only attenuated the ADP-evoked intracellular Ca^{2+} rise by approximately 16–19%, whereas EGTA nearly abolished the extracellularly supported component. This pattern indicates that scutellarin does not act by indiscriminate calcium sequestration in the assay medium, but rather by modulating signaling pathways that govern Ca^{2+} mobilization and/or Ca^{2+} entry.

A mechanistically plausible interpretation is that scutellarin affects receptor-coupled phospholipase C signaling, intracellular store release, PKC-linked feedback, or store-operated Ca^{2+} entry (SOCE). In platelets, depletion of internal Ca^{2+} stores is sensed by

STIM1, which activates Orai1-containing channels and sustains cytosolic Ca^{2+} elevation. High and sustained cytosolic Ca^{2+} is particularly important for the transition from a purely aggregatory platelet response to a procoagulant phenotype with phosphatidylserine exposure and accelerated tenase/prothrombinase assembly. Therefore, even a moderate reduction in agonist-evoked Ca^{2+} signaling may have disproportionately large effects on secondary aggregation, dense-granule reinforcement, and procoagulant membrane expression. This provides a coherent explanation for why scutellarin only modestly reduced the Ca^{2+} transient yet strongly suppressed ADP-dependent aggregation and globally prolonged clotting-based assays.

The observation that heparin was associated with a modest increase in intracellular Ca^{2+}, whereas scutellarin suppressed the ADP-triggered rise, further emphasizes mechanistic divergence between the two compounds. Heparin is primarily an antithrombin-dependent anticoagulant and is not classically regarded as a direct platelet Ca^{2+}-signaling suppressor. Scutellarin, in contrast, appears to express a mixed pharmacological signature: it shifts plasma coagulation toward hypocoagulability while simultaneously weakening platelet Ca^{2+}-dependent activation. On the basis of the currently available functional data, the most conservative interpretation is that scutellarin acts as a multitarget antithrombotic modulator with convergent effects on thrombin/fibrin kinetics and platelet signaling rather than as a simple ion chelator or a single-enzyme inhibitor.

Integrated mechanistic interpretation

Taken together, the data support a multilevel antithrombotic model for scutellarin. At the plasma level, prolongation of TT, PT, APTT, and recalcification time indicates a broad slowing of coagulation kinetics involving the terminal thrombin–fibrin step and membrane-dependent propagation reactions. At the cellular level, inhibition of ADP-induced aggregation together with attenuation of intracellular Ca^{2+} signaling points to suppression of platelet amplification pathways that normally stabilize $\alpha IIb\beta_3$ activation, dense-granule–dependent reinforcement, and procoagulant phosphatidylserine exposure. Because related scaffold data implicate PKC-centered platelet signaling and because phosphatidylserine-dependent tenase/prothrombinase assembly is highly sensitive to sustained cytosolic Ca^{2+}, the most scientifically defensible conclusion is that scutellarin does not behave as a simple chelator, but rather as a multitarget antithrombotic modulator acting at the interface of platelet activation and plasma coagulation. Direct enzyme-kinetic confirmation against thrombin, factor Xa, and platelet signaling nodes would be the logical next step for mechanistic validation.

DISCUSSION

The present findings support the interpretation that scutellarin exerts a multilevel antithrombotic effect involving both plasma coagulation and platelet-dependent signaling. The most prominent signal in the coagulation panel was the marked prolongation of thrombin time, accompanied by prolongation of activated recalcification time, APTT, and PT. Considered together, these changes argue against a narrowly pathway-restricted action and instead suggest a broader slowing of clot formation kinetics. Because thrombin time primarily reflects the conversion of fibrinogen to fibrin after addition of exogenous thrombin, TT prolongation is classically interpreted as interference with thrombin catalytic function, thrombin–fibrinogen interaction, or fibrin polymerization rather than isolated inhibition of early contact activation [19]. The absence of a clear effect when fibrinogen alone was preincubated under the reported conditions further suggests that gross fibrinogen destabilization is unlikely to be the sole explanation. Rather, the data are more consistent with modulation of the terminal thrombin–fibrin step or closely related catalytic events that determine the efficiency of fibrin clot formation [19].

The concomitant prolongation of APTT and PT reinforces this interpretation. APTT reflects intrinsic/common pathway kinetics, whereas PT is more sensitive to the extrinsic/common axis; simultaneous prolongation of both therefore usually indicates a broader reduction in overall coagulation efficiency rather than inhibition of only one classical branch of the cascade [1]. In the current study, this pattern, together with the strong TT response, suggests that scutellarin may influence coagulation at multiple levels, including factor activation dynamics, common-pathway propagation, and final thrombin-driven fibrin formation. Such a profile is compatible with the broader pharmacological behavior reported for the scutellarin/scutellarein scaffold, in which thrombin-oriented clotting assays, including TT, PT, and APTT, were used to identify anticoagulant activity and structure–activity relationships among related flavone derivatives [20]. Although those data do not by themselves prove that native scutellarin directly binds thrombin or factor Xa in the present system, they support the plausibility of a scaffold-level anticoagulant phenotype.

The prolongation of activated recalcification time adds an important mechanistic dimension. Recalcification assays are strongly dependent on restoration of ionized

Ca^{2+} and on efficient assembly of coagulation enzyme complexes on negatively charged phospholipid surfaces. In cell-based hemostasis, vitamin K-dependent factors are recruited to phosphatidylserine-rich membranes through Ca^{2+}-dependent interactions, enabling rapid assembly of intrinsic tenase and prothrombinase [1]. Therefore, prolongation of recalcification time is best interpreted not simply as "slower clotting," but as evidence that the membrane-dependent organization of coagulation may be less efficient in the presence of scutellarin. This is mechanistically compatible with reduced phosphatidylserine-supported complex assembly, weaker anchoring of vitamin K-dependent factors, or suppression of the platelet procoagulant surface that normally accelerates thrombin generation [1,13]. In this respect, the recalcification data link the plasma assays to the platelet findings and suggest that scutellarin may act at the interface of cellular and plasmatic hemostasis.

The platelet results strengthen this integrated interpretation. Scutellarin markedly inhibited ADP-induced aggregation and, according to the source data, almost abolished the second phase of aggregation. This distinction is mechanistically meaningful because ADP-driven platelet activation is not a single-step event. The early phase is largely associated with $P2Y_1$-linked Ca^{2+} mobilization and shape change, whereas stable and amplified aggregation depends on sustained signaling through $P2Y_{12}$, secondary secretion, and persistent integrin $\alpha IIb\beta_3$ activation [21]. Thus, near-complete suppression of the second wave is more consistent with interference in ADP-dependent amplification than with a simple reduction of initial platelet contact responses. The finding that the inhibitory effect became more evident in the presence of extracellular Ca^{2+} also supports a signaling-based mechanism, because extracellular Ca^{2+} contributes to the persistence and stability of ADP-driven platelet activation rather than serving merely as an inert background ion [21].

The intracellular Ca^{2+} data help refine this mechanism. Scutellarin attenuated the ADP-evoked rise in platelet cytosolic Ca^{2+}, but the magnitude of inhibition was clearly smaller than that produced by EGTA, indicating that scutellarin does not behave as a simple extracellular Ca^{2+} chelator. Instead, the data are more compatible with partial interference in receptor-coupled Ca^{2+} handling, intracellular store mobilization, or store-operated Ca^{2+} entry. In platelets, sustained cytosolic Ca^{2+} signals are critically dependent on the STIM1–Orai1 axis, and disruption of this pathway impairs procoagulant activity and thrombus formation [13, 22-25]. Moreover, phosphatidylserine exposure on activated platelets depends on strong and sustained Ca^{2+} elevation; this surface remodeling then supports assembly of intrinsic tenase and prothrombinase, thereby accelerating thrombin generation [13]. From this perspective, even a moderate reduction in agonist-evoked Ca^{2+} signaling may have disproportionate downstream consequences for secondary aggregation, procoagulant membrane expression, and thrombin output. The current data fit well with that model.

A useful mechanistic clue comes from the related aglycone scutellarein, which is recognized as a principal in vivo metabolite of scutellarin. In rat washed platelets, scutellarein inhibited platelet adhesion and aggregation induced by ADP, thrombin, and U46619, while exerting only a mild effect on intracellular Ca^{2+} mobilization; importantly, that study identified protein kinase C (PKC) as a likely intracellular target [18, 26-28]. This observation is highly relevant here because it suggests that the scutellarin/scutellarein scaffold may not act by blocking only one membrane receptor or by indiscriminate Ca^{2+} scavenging, but rather by dampening intracellular amplification pathways downstream of agonist binding. The current dataset-strong suppression of ADP aggregation, only partial attenuation of the Ca^{2+} transient, and broad prolongation of coagulation assays-is fully compatible with such a mechanism. In other words, scutellarin appears more likely to function as a signaling modulator than as a simple stoichiometric inhibitor of extracellular calcium.

When the coagulation and platelet data are interpreted together, a coherent model emerges. Scutellarin appears to weaken thrombus formation at two mutually reinforcing levels: first, by reducing the efficiency of plasma coagulation as reflected in TT, PT, APTT, and recalcification assays; and second, by suppressing platelet amplification processes that are necessary for sustained aggregation and for generation of a procoagulant phosphatidylserine-positive surface. Because platelet procoagulant activity and plasma coagulation are tightly coupled in cell-based hemostasis, simultaneous partial effects at both levels may translate into a stronger net antithrombotic phenotype than would be expected from either effect in isolation [1,6,13,29,30]. This integrative interpretation also explains why the observed functional anticoagulation does not require the compound to behave like a direct chelator or a highly selective single-enzyme inhibitor.

At the same time, the present data should be interpreted with appropriate caution. The clotting assays demonstrate a clear functional hypocoagulant phenotype, but they do not by themselves identify a unique molecular target. Likewise, suppression of ADP-induced aggregation and attenuation of intracellular

Ca^{2+} signaling indicate interference with platelet activation pathways, but they do not prove direct binding to P2Y receptors, Orai1/STIM1, PKC, thrombin, or factor Xa. Accordingly, the most scientifically defensible conclusion is that scutellarin acts as a multitarget antithrombotic modulator, with convergent effects on platelet signaling and plasma coagulation, rather than as a simple chelator or a pathway-exclusive inhibitor. Future work should therefore focus on direct enzyme-kinetic assays against thrombin and factor Xa, platelet phosphatidylserine quantification, thrombin generation analysis, and targeted interrogation of PKC- and SOCE-related signaling nodes.

From a translational standpoint, this multitarget profile may be particularly relevant. Single-target antithrombotic strategies are often effective but may be limited by pathway escape or by a narrow therapeutic window. A compound that modestly attenuates both platelet activation and coagulation propagation could, at least in principle, provide balanced antithrombotic activity through distributed inhibition across the hemostatic network. Whether scutellarin can achieve such an effect safely in vivo remains to be established, but the present results justify further investigation of this flavone scaffold as a candidate modulator at the interface of platelet biology and coagulation.

CONCLUSION

In conclusion, the present study demonstrates that scutellarin expresses a coherent multilevel antithrombotic profile in vitro and ex vivo. Functionally, scutellarin prolonged thrombin time, activated recalcification time, APTT, and PT, indicating that its anticoagulant activity is not limited to a single classical branch of the coagulation cascade but extends across intrinsic/extrinsic propagation and the terminal thrombin–fibrin step. In parallel, scutellarin markedly inhibited ADP-induced platelet aggregation and attenuated the agonist-evoked rise in intracellular Ca^{2+}. Because this Ca^{2+} effect was clearly weaker than that of EGTA, scutellarin should not be interpreted as a simple extracellular calcium chelator. Rather, the overall pattern is more consistent with interference in platelet amplification pathways, receptor-coupled signaling, membrane-dependent coagulation-complex assembly, or closely related thrombin-generating processes. Taken together, these findings support the view that scutellarin acts at the interface of platelet activation and plasma coagulation, thereby reducing thrombus-forming potential through convergent functional mechanisms. At the same time, the current data remain principally phenotypic and do not identify a unique molecular target. Further work should therefore include direct enzyme-kinetic studies against thrombin and factor Xa, assessment of phosphatidylserine exposure and thrombin generation, and in vivo validation in standardized thrombosis models to define the translational antithrombotic potential of scutellarin more precisely.

Graphical Abstract Caption

Scutellarin exerts a multitarget antithrombotic effect by attenuating platelet activation and slowing plasma coagulation. In the present study, scutellarin prolonged TT, activated recalcification time, APTT, and PT, indicating reduced coagulation efficiency at the levels of thrombin–fibrin conversion and pathway propagation. Simultaneously, it inhibited ADP-induced platelet aggregation and attenuated agonist-evoked intracellular Ca^{2+} elevation without behaving as a simple calcium chelator. The integrated functional profile is therefore consistent with modulation of both platelet signaling and membrane-dependent coagulation processes, supporting scutellarin as a candidate antithrombotic modulator acting across the platelet–coagulation interface.

Scutellarin reduced thrombus-related functional activity through concurrent inhibition of platelet amplification and plasma coagulation, supporting a multitarget antithrombotic mechanism rather than a single-pathway effect.

Suggested Highlights

Scutellarin markedly prolonged TT, APTT, PT, and activated recalcification time.

Scutellarin strongly inhibited ADP-induced platelet aggregation.

Scutellarin attenuated agonist-evoked platelet Ca^{2+} rise without EGTA-like chelation.

The data support a multitarget antithrombotic effect at the platelet–coagulation interface.

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