Jurnal Pembelajaran Dan Biologi Nukleus p-ISSN: 2442-9481
Vol 12 (1): 213 – 227, March 2026 e-ISSN: 2685-7332

Phytochemical Profiling and Hemostatic Potential of *Iris domestica* Fruit Extract for Wound Bleeding Control

Jon Kenedy Marpaung[1], Manuppak Irianto Tampubolon[1], Adiansyah Adiansyah (*)[2]

[1] Department of Pharmacy, Faculty of Pharmacy and Health Sciences, Sari Mutiara Indonesia University
[2] Department of Chemistry, Faculty of Science and Technology, Sari Mutiara Indonesia University
Jl. Kapten Muslim No.79, Medan Helvetia, Medan City, Sumatera Utara 20124, Indonesia

***Corresponding Author:** adiansyah_skd@yahoo.co.id

Submitted November 16 Th 2025, and Accepted February 10 Th 2026

Abstract

Background: *Uncontrolled bleeding remains a major challenge in wound care, particularly in emergency and resource-limited settings. Limitations of synthetic hemostatic agents, including high cost, side effects, and limited access, have driven interest in plant-based alternatives. Iris domestica contains bioactive metabolites with astringent and wound-healing properties. This study aimed to characterize its phytochemical constituents and assess the hemostatic activity of its fruit extract.* **Methodology:** *Phytochemical screening was conducted on powdered simplicia, ethanol extract, and solvent fractions (n-hexane, ethyl acetate, and aqueous). Antibacterial activity was evaluated using the agar well diffusion method against Gram-positive and Gram-negative bacteria. Hemostatic activity was assessed through in vitro clotting time and in vivo bleeding time assays in rodents, grouped into negative control (saline), positive control (standard agent), and extract-treated groups at varying concentrations. Data were analyzed using one-way ANOVA with Tukey's post hoc test ($p < 0.05$).* **Findings:** *Phytochemical analysis showed that the ethanol extract and ethyl acetate fraction contained alkaloids, flavonoids, glycosides, saponins, and tannins, with no triterpenoids detected. The ethanol extract exhibited dose-dependent antibacterial activity, with the highest inhibition against Staphylococcus aureus (12.70 mm at 250 mg/mL). Hemostatic assays revealed significantly reduced clotting and bleeding times compared to the negative control ($p < 0.05$), with activity comparable to the positive control, indicating strong hemostatic potential.* **Contributions:** *I. domestica fruit extract exhibits significant hemostatic activity, likely mediated by tannins via protein precipitation and vasoconstriction, with synergistic contributions from alkaloids and saponins. The inclusion of appropriate controls strengthens this evidence. These findings highlight its potential as a natural alternative to conventional hemostatic agents and warrant further bioassay-guided isolation and safety evaluation.*

Keywords: *Antibacterial activity; Hemostatic agent; Iris domestica; Phytochemical screening; Wound healing*

https://doi.org/10.36987/jpbn.v12i1.8460

INTRODUCTION

Despite advances in biomaterials and pharmacologic interventions, current research on natural hemostatic agents remains fragmented and largely preliminary. Several plant-derived compounds—such as tannin-rich extracts from *Vitis vinifera, Camellia sinensis,* and *Hamamelis virginiana* have demonstrated promising in vitro clotting enhancement and in vivo bleeding control, often attributed to protein precipitation and vasoconstrictive effects (Theisen et al., 2014; Ebrahimi et al., 2020; Marcińczyk et al., 2022). However, many of these studies are limited by narrow phytochemical characterization, lack of robust comparative controls, or evaluation in a single bleeding model, which constrains their translational relevance. Moreover, the majority of explored extracts focus on leaf or bark tissues, with relatively few investigations into fruit matrices, which may harbor distinct metabolite profiles with unique bioactivities. To date, there is limited evidence on the hemostatic potential of *Iris domestica* fruit, despite its ethnopharmacological use and rich metabolite profile reported in phytochemical surveys. This study addresses these gaps by providing comprehensive phytochemical analysis, systematic evaluation across multiple hemostatic assays (both in vitro clotting time and in vivo bleeding time), and inclusion of both negative and positive controls for comparative benchmarking. In doing so, it positions the investigation at the intersection of traditional knowledge and rigorous biomedical evaluation, offering novel insights into the hemostatic utility of *Iris domestica* fruit extract relative to existing natural and synthetic agents.

Uncontrolled bleeding remains one of the leading causes of preventable death in trauma, surgery, and certain coagulopathic conditions worldwide (Montazerian et al., 2022). Effective hemostasis is critical not only to reduce mortality but also to accelerate the wound-healing process and prevent secondary complications. Conventional hemostatic products such as fibrin sealants, thrombin-based agents, and synthetic polymer dressings have demonstrated clinical efficacy. However, these products are often associated with significant drawbacks, including high cost, storage instability, immunogenic responses, or the potential risk of pathogen transmission in biologically derived materials (Guo et al., 2023a). These limitations have driven a strong interest in developing novel, safe, biocompatible, and cost-effective hemostatic alternatives from natural sources.

Medicinal plants are a well-established reservoir of bioactive compounds with therapeutic potential. Numerous phytochemicals have been reported to exhibit hemostatic properties by enhancing coagulation pathways, inducing vasoconstriction, modulating platelet aggregation, or exerting protein-precipitating astringent effects (Ebrahimi et al., 2020). For example, tannins are known for their strong astringency, enabling local vasoconstriction and protein coagulation at the wound site, while alkaloids may act as vasomodulators with potential pro-coagulant activity. Flavonoids help stabilize vascular integrity, reduce capillary permeability, and promote platelet aggregation. Saponins, due to their amphiphilic nature, can interact with cell membranes and facilitate platelet adhesion, while glycosides may influence cellular signaling related to coagulation and tissue repair (Samavati et al., 2025). The synergistic activity of these phytochemicals makes plants an attractive and underexplored source for developing hemostatic agents.

Among these plants, *Iris domestica* (L.) Goldblatt & Mabb, previously classified as *Belamcanda chinensis*, has gained increasing scientific interest. Belonging to the family Iridaceae, the species is widely distributed in Asia and has been traditionally used in ethnomedicine to treat inflammatory conditions, infections, and wound-related ailments. In Indonesia, the fruit of *I. domestica* is locally known as *Iris domestica* (L.) Goldblatt & Mabb. Modern phytochemical investigations of Iris species confirm the presence of diverse secondary metabolites, including isoflavonoids, xanthones, stilbenoids, triterpenoids, glycosides, tannins, alkaloids, and saponins (Khatib et al., 2022). Many of these compounds demonstrate pharmacological activities relevant to wound management, such as anti-inflammatory, antioxidant, antimicrobial, and cytoprotective effects (Badran et al., 2021).

Recent studies have highlighted the therapeutic potential of *I. domestica*. Isoflavonoids such as tectorigenin and irigenin, isolated from its rhizome and fruit, have demonstrated promising anti-inflammatory activity, with related flavonoid glucosides (e.g., tectoridin A) inhibiting the NF-κB signaling pathway by ~53.7% at 10 μM, and other analogs showing up to ~88.7% inhibition at the same concentration in in vitro reporter assays, indicating significant suppression of pro-inflammatory signaling. Additionally, phenolic constituents exhibited notable cytotoxic activity; for example, 4′-O-methylnyasol showed ~84.9% antiproliferative activity against K562 leukemia cells at 10 μM. While specific IC_{50} values for antioxidant action of tectorigenin and irigenin in *I. domestica* remain less defined, related Iris species report strong radical scavenging and lipid peroxidation inhibition in DPPH/ABTS assays. Despite these findings, relatively little attention has been given to their role in hemostasis, representing a clear research gap given the plant's traditional wound-healing use and phytochemical rationale for coagulation activity. In addition, cytotoxic compounds isolated from *I. domestica* have shown pro-apoptotic activity against cancer cells, underscoring the pharmacological richness of this species (Guo et al., 2023b). While these studies emphasize its potential in cancer and anti-inflammatory research, relatively little attention has been given to its possible role in hemostasis. This research gap is particularly striking, given the traditional use of the plant for treating wounds and the strong phytochemical rationale for its hemostatic potential (Farag, et.al., 2009).

Preliminary phytochemical screening of *I. domestica* fruit extracts has demonstrated the presence of tannins, flavonoids, glycosides, saponins, and alkaloids, while triterpenoids and steroids are absent (Marpaung et al., 2020). This composition is of particular interest because tannins and flavonoids are strongly correlated with hemostatic activity. Tannins exert vasoconstrictive and protein-precipitating effects, which directly reduce bleeding, while flavonoids and alkaloids enhance vascular stability and clot formation. Meanwhile, saponins may promote platelet adhesion and aggregation, which are essential steps in primary hemostasis (Ebrahimi et al., 2020).

In addition to their hemostatic potential, extracts of *I. domestica* demonstrate antimicrobial activity against clinically relevant wound pathogens. Antimicrobial testing against *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Salmonella typhi,* and *Klebsiella pneumoniae* has shown concentration-dependent inhibition zones, with *S. aureus* being the most sensitive species

(Marpaung et al., 2020). These results suggest a dual function for *I. domestica*: not only controlling bleeding but also preventing wound infection, which is a major complication in wound care and a factor that delays healing. This dual action represents a significant advantage over many conventional hemostatic agents, which lack intrinsic antimicrobial activity.

Despite these promising properties, systematic studies investigating the hemostatic mechanisms of *I. domestica* remain limited. Existing research has focused primarily on its phytochemical composition and general pharmacological activities. There is a clear need for integrated studies combining phytochemical characterization, antimicrobial evaluation, and hemostatic assays to establish a mechanistic link between the bioactive constituents and their potential clinical applications. In particular, studies should employ in vitro clotting assays, platelet aggregation tests, and in vivo bleeding time models to validate the plant's effectiveness as a natural hemostatic agent (Schmid, *et.al*., 2021).

The present research addresses this gap by characterizing the phytochemical constituents of *I. domestica* fruit extract and its solvent fractions, evaluating their antimicrobial activity against wound-associated pathogens, and investigating their hemostatic potential through clotting assays and bleeding models. By correlating the phytochemical profile with observed biological activity, this study aims to provide robust scientific evidence for the development of *I. domestica* as a plant-derived hemostatic agent. Ultimately, the findings are expected to contribute to the advancement of affordable, safe, and effective natural alternatives for bleeding control and wound management, particularly in resource-limited settings.

METHOD

Plant Material Collection and Authentication

Fresh fruits of *I. domestica* (L.) Goldblatt & Mabb were collected in June 2024 from Padang Pulau Village, Bandar Pulau Subdistrict, Asahan Regency, North Sumatra Province, Indonesia. Plant material collection was conducted using a purposive sampling method, targeting mature and healthy plants traditionally recognized by local communities for medicinal use. The plant material was taxonomically authenticated by a botanist at the Herbarium (Universitas Sumatera Utara Herbarium), Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia. The collected fruits were washed with running water, air-dried under shade at room temperature (25–27 °C) for 14 days, and subsequently pulverized into coarse powder using a mechanical grinder prior to extraction.

Preparation of Plant Extracts and Fractions

The dried fruit powder (500 g) was macerated in 96% ethanol at a ratio of 1:10 (w/v) for 72 h at room temperature with intermittent shaking. The mixture was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C to obtain the crude ethanol extract. The extraction yield was calculated as the percentage of dried extract relative to the initial dry plant material. For fractionation 50 g of the crude ethanol extract was suspended in distilled water

and subjected to liquid–liquid partitioning using solvents of increasing polarity, namely n-hexane, ethyl acetate, and water. Partitioning with each solvent was performed repeatedly (3 ×) using an equal volume of solvent until the organic phase became colorless, ensuring exhaustive extraction of compounds according to polarity by BPOM RI (2019). The combined solvent layers were separately concentrated under reduced pressure to obtain the n-hexane, ethyl acetate, and aqueous fractions, which were dried to constant weight. Each fraction was weighed, and the fraction yields were calculated as the percentage relative to the weight of the crude ethanol extract used for fractionation. All extracts and fractions were stored at 4 °C until further use (Sun et al., 2025).

Phytochemical Screening

Qualitative phytochemical screening was performed on powdered simplicia, crude ethanol extract, and solvent fractions following standard procedures described by Harborne (1998), with minor modifications based on updated protocols (Shaikh & Patil, 2020). The modifications primarily involved adjustments in sample concentration, solvent system, and reaction conditions to improve sensitivity and clarity of color development. Briefly, all extracts and fractions were dissolved in their respective compatible solvents at a concentration of 10 mg/mL, while powdered simplicia was prepared as an aqueous or ethanolic decoction prior to testing. For alkaloid detection, both Dragendorff's and Mayer's reagents were applied after acidification with dilute hydrochloric acid to enhance alkaloid solubility (Demarque et al., 2020). The Shinoda test for flavonoids was conducted using magnesium turnings and concentrated hydrochloric acid with extended reaction time (5 min) to ensure stable color formation. Glycosides were detected using the Keller–Killiani test with careful layering of reagents to improve ring visibility. Saponins were identified using the frothing test with prolonged shaking (30 s) and a 15-min standing period to confirm froth stability. Tannins were detected using 1% ferric chloride solution, and the Liebermann–Burchard test was employed for triterpenoids/steroids using freshly prepared reagents (Bonfils et al., 2021).

Antimicrobial Activity Assay

The antimicrobial activity of the crude ethanol extract and solvent fractions was evaluated against five clinically relevant wound pathogens: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 14028, and *Klebsiella pneumoniae* ATCC 13883. The agar well diffusion method was employed following CLSI guidelines. Standardized bacterial suspensions (0.5 McFarland standard; approximately 1.5×10^8 CFU/mL) were uniformly swabbed onto Mueller–Hinton agar plates. Wells of 6 mm diameter were filled with 100 µL of extract or fraction at concentrations ranging from 12.5 to 500 mg/mL.

Each experiment was performed in triplicate (n = 3), and the results were expressed as mean inhibition zone diameter ± standard deviation. Ciprofloxacin (10 µg/disc) was used as the positive control, while 1% DMSO served as the negative control. Plates were incubated at 37 °C for 24 h, and inhibition zones were measured

in millimetres. The agar well diffusion assay was applied as a semi-quantitative screening method to evaluate antimicrobial potential based on diffusion-dependent growth inhibition. This study did not include minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) determination, which is acknowledged as a limitation and warrants further investigation using broth dilution methods. The use of relatively high extract concentrations (up to 500 mg/mL) was based on established practices in preliminary antimicrobial screening of crude plant extracts, which typically require higher doses due to the complex mixture of active and inactive constituents and limited diffusion capacity in agar media (Balouiri et al., 2016).

Hemostatic Activity Evaluation

In Vitro Clotting Assay

The effect of the crude extract and solvent fractions on blood clotting time was evaluated using the capillary tube method, with minor modifications from (Lewis & Glueck, 1958). Fresh citrated human blood was obtained from healthy adult volunteers after written informed consent, in accordance with ethical approval from the Institutional Ethics Committee of Sari Mutiara Indonesia University Briefly, blood samples were equilibrated to room temperature prior to analysis. A fixed volume of blood was mixed with the test samples at concentrations ranging from 50 to 500 mg/mL using a blood-to-sample ratio of 9:1 (v/v), which is commonly applied to minimize dilution effects while allowing sufficient interaction between bioactive constituents and coagulation components. For controls, normal saline served as the negative control, while calcium chloride solution (0.025 M) was used as the positive control to reverse citrate anticoagulation and initiate coagulation. In selected assays, topical thrombin was employed as an additional reference hemostatic agent. Clotting time was recorded as the interval between sample addition and the first appearance of a visible fibrin thread inside the capillary tube. All experiments were performed in triplicate (Cheng at al.,2019).

In Vivo Tail Bleeding Model in Mice

Male Swiss albino mice (25–30 g) were acclimatized for one week under standard laboratory conditions and randomly assigned into experimental groups (n = 5 per group). Randomization was performed using a simple random allocation method, in which animals were assigned to groups based on a computer-generated random number sequence to minimize selection bias. Animals were anesthetized using a ketamine/xylazine combination (ketamine 100 mg/kg and xylazine 10 mg/kg, intraperitoneally). After achieving adequate anesthesia, approximately 5 mm of the distal tail tip was transected using a sterile surgical blade. The wound area was immediately treated with the test samples by topical application of 50 µL of extract or fraction solution at the designated concentration using sterile cotton swabs.

The negative control group received sterile normal saline, while the positive control group was treated with a commercial oxidized regenerated cellulose pad, a commonly used topical hemostatic agent. Bleeding time was recorded as the interval from tail incision until complete cessation of bleeding, defined as the absence of visible blood flow for at least 30 seconds. All measurements were performed by the same

observer to reduce inter-observer variability. All animal experimental procedures were conducted in accordance with the ARRIVE guidelines and were approved by the Animal Ethics Committee of Universitas Sari Mutiara Indonesia.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test to compare treatment groups. A value of $p < 0.05$ was considered statistically significant. Analyses were performed using GraphPad Prism 10.

RESULT AND DISCUSSION

Characterization of *Iris domestica* (L.) Goldblatt & Mabb Simplisia

The characterization of fruit simplisia was conducted to ensure its quality, safety, and compliance with pharmacopeial standards prior to phytochemical investigation. The results demonstrated that all evaluated parameters met the acceptance criteria recommended by the Indonesian Herbal Pharmacopoeia. The moisture content of the simplisia was found to be 8.60%, which is below the maximum permissible limit of 10%. Low moisture content is a critical quality attribute, as it minimizes the risk of microbial and fungal growth during storage, thereby enhancing the stability and shelf life of the raw material. This finding indicates that the drying process applied to the *Iris domestica* (L.) Goldblatt & Mabb fruit was effective and appropriate (Kumaraswamy et.al., 2008).

Table 1. Physicochemical Characterization of *Iris domestica* (L.) Fruit Simplisia

No	Characterization Parameter	Test Result (%)	Reference Standard (Pharmacopoeia/ Guideline)	Interpretation
1	Moisture content	8.60	< 10% (Indonesian Herbal Pharmacopoeia)	Complies with the standard; low moisture content minimizes the risk of microbial and fungal growth and improves storage stability.
2	Water-soluble extractive value	14.65	> 10% (WHO Guidelines on Quality Control Methods for Herbal Materials)	Complies with the standard; indicates a high content of polar metabolites such as flavonoids and glycosides soluble in water.
3	Ethanol-soluble extractive value	9.58	> 7% (Indonesian Herbal Pharmacopoeia)	Complies with the standard; reflects the presence of semi-polar to non-polar compounds including

No	Characterization Parameter	Test Result (%)	Reference Standard (Pharmacopoeia/ Guideline)	Interpretation
				alkaloids and phenolic constituents.
4	Total ash content	3.33	< 12% (Indonesian Herbal Pharmacopoeia)	Complies with the standard; indicates normal levels of inorganic matter and absence of excessive contamination.
5	Acid-insoluble ash content	0.26	< 1% (Indonesian Herbal Pharmacopoeia)	Complies with the standard; suggests minimal contamination with siliceous materials such as sand or soil.

Table 2. Qualitative Phytochemical Screening of *Iris domestica* (L.) Fruit Simplisia, Ethanol Extract, and Fractions

No.	Phytochemical Class	Simplisia Powder	Ethanol Extract	n-Hexane Fraction	Ethyl Acet Fraction	Residual Fraction
1	Alkaloids	+	+	–	+	–
2	Flavonoids	+	+	–	+	+
3	Glycosides	+	+	–	+	+
4	Saponins	+	+	–	+	+
5	Tannins	+	+	–	+	–
6	Triterpenoids/ Steroids	–	–	–	–	–

Note: (+) indicates the presence of the phytochemical class as determined by qualitative screening tests, while (–) indicates absence or non-detectable levels under the experimental conditions.

The water-soluble extractive value was 14.65%, exceeding the minimum requirement of 10%. This high value suggests a substantial presence of polar secondary metabolites, such as flavonoids, glycosides, saponins, and other hydrophilic constituents. Such compounds are often associated with antioxidant, anti-inflammatory, and antimicrobial activities, indicating the therapeutic potential of *Iris domestica* (L.) fruit. The ethanol-soluble extractive value was recorded at 9.58%, which is higher than the minimum standard of 7%. This result reflects the presence of semi-polar to non-polar bioactive compounds, including alkaloids, phenolic compounds, and terpenoid-related constituents. The ability of ethanol to extract a broad range of phytochemicals highlights its suitability as a solvent for further pharmacological and phytochemical studies (Hayet et al., 2008). The total ash content of the simplisia was 3.33%, well below the acceptable limit of 12%. This low ash value indicates a normal level of inorganic and mineral content and suggests the absence of excessive contamination with extraneous matter. Furthermore, the acid-insoluble ash content was 0.26%, which is significantly lower than the maximum limit of 1%. This result

confirms minimal contamination with siliceous materials such as sand or soil, supporting the purity of the raw material (Khanna et al., 2008). Overall, the characterization data confirm that *Iris domestica* (L.) fruit simplisia possesses good physicochemical quality and fulfills pharmacopeial requirements, making it suitable for further extraction and phytochemical evaluation (Ballester et.al.,2022).

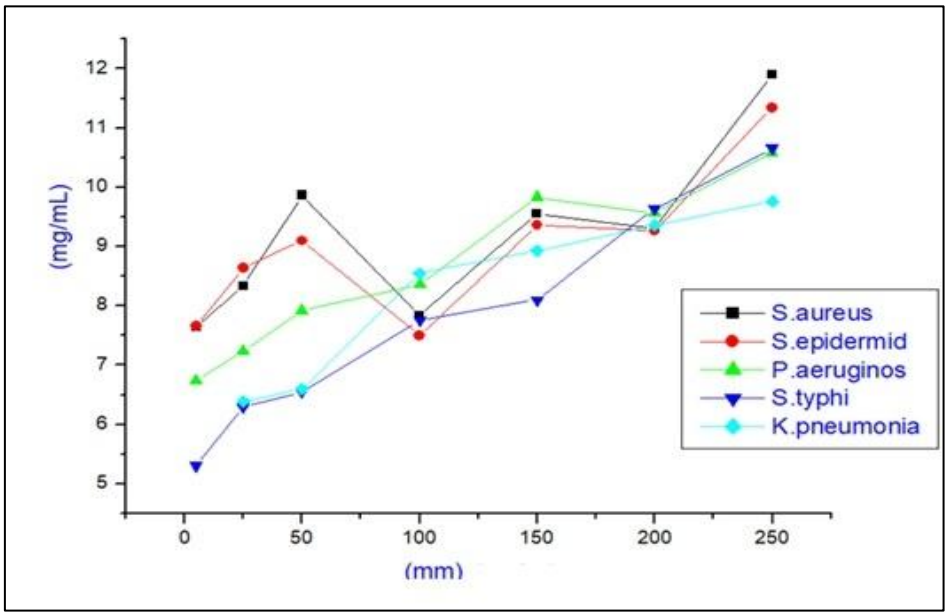
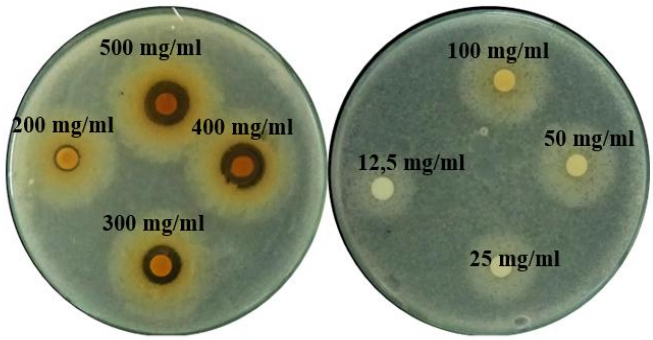
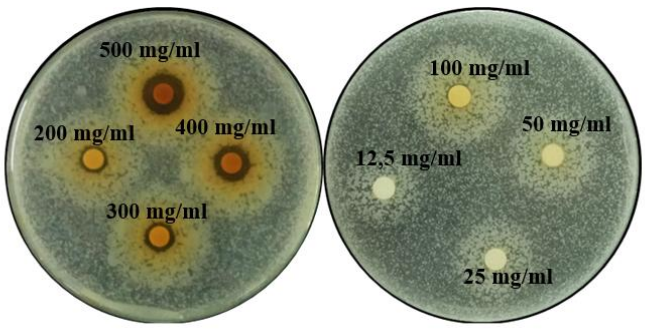


Figure 1. Antibacterial activity of the ethanolic extract of *Iris domestica* fruit against selected wound-associated pathogenic bacteria, expressed as inhibition zone diameters at different extract concentrations.



A



B

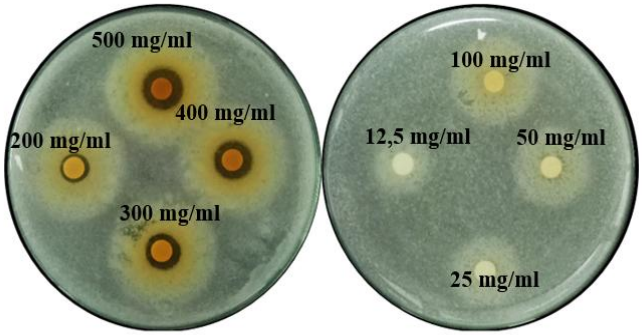


Figure 2. Antibacterial Activity Results of the Residual Fraction of *Iris domestica* Fruit

Phytochemical Screening of Simplisia, Ethanol Extract, and Fractions

Qualitative phytochemical screening was performed on the simplisia powder, ethanol extract, and various solvent fractions (n-hexane, ethyl acetate, and residual fraction) to identify the distribution of secondary metabolites based on polarity. The simplisia powder and ethanol extract showed positive results for alkaloids, flavonoids, glycosides, saponins, and tannins, indicating that these major classes of bioactive compounds are naturally present in *Iris domestica* (L.) fruit. The consistency between simplisia and ethanol extract profiles suggests that ethanol effectively extracts most of the phytochemical constituents from the raw material (Amarowicz et.al., 2005). In the n-hexane fraction, none of the tested phytochemical groups were detected. This finding indicates a low abundance or absence of non-polar compounds such as lipophilic terpenoids or steroids in *Iris domestica* (L.) fruit, which is consistent with the negative results observed for triterpenoid/steroid tests across all samples (Ayustaningwarno et al., 2004).

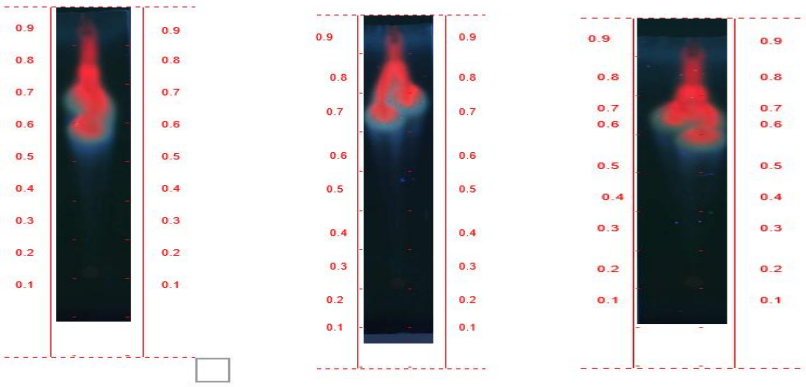
The ethyl acetate fraction exhibited positive reactions for alkaloids, flavonoids, glycosides, saponins, and tannins. This fraction demonstrated the richest phytochemical profile among the fractions, suggesting that the majority of bioactive compounds in *Iris domestica* (L.) fruit are semi-polar in nature. Ethyl acetate is well known for its effectiveness in concentrating phenolic and flavonoid compounds, which are often responsible for significant biological activities (BPOM, 2000).

The residual (aqueous) fraction showed positive results for flavonoids, glycosides, and saponins, while alkaloids and tannins were not detected. This distribution further supports the predominance of polar constituents in the fruit and aligns with the high water-soluble extractive value obtained during characterization. Notably, triterpenoids/steroids were not detected in any sample, indicating that these compounds are either absent or present in concentrations below the detection limit of the applied qualitative tests (Amarowicz et.al., 2005).

Implications for Pharmaceutical Research

The presence of multiple bioactive secondary metabolites, particularly flavonoids, alkaloids, glycosides, saponins, and tannins, supports the potential of *Iris domestica* (L.) fruit as a promising source of pharmacologically active compounds. The dominance of polar and semi-polar constituents suggests potential antioxidant,

antimicrobial, and anti-inflammatory properties, which merit further quantitative analysis and bioactivity-guided fractionation. In conclusion, the physicochemical characterization and phytochemical screening confirm that *Iris domestica* (L.) fruit simplisia meets international quality standards and contains diverse secondary metabolites with potential pharmaceutical applications (Atanasov et al., 2001). These findings provide a scientific basis for further pharmacological, toxicological, and standardization studies aimed at developing *Iris domestica* (L.) as a herbal medicinal resource (Bandyopadhyay et al., 2025).



(a) (b) (c)

Figure 3. Paper Chromatographic Profile of the Ethyl Acetate Fraction of *Iris domestica* Fruit

The image presents a thin-layer chromatographic (TLC) profile of *Iris domestica* extract/fraction visualized under UV light at 366 nm using a CAMAG Visualizer system. The chromatogram displays several distinct fluorescent bands with red–orange emission distributed along the silica gel plate. Prominent zones are observed at moderate to high Rf values (approximately 0.6–0.8), accompanied by diffuse bands, suggesting the presence of multiple constituents. The dark background of the plate provides good contrast, allowing clear visualization of fluorescent compounds without background interference (Siafaka et al., 2025).

The appearance of intense red–orange fluorescence under UV 366 nm is characteristic of phenolic compounds with extended conjugation systems, particularly flavonoids and isoflavonoids, which are known phytochemical markers of *I. domestica*. The presence of multiple bands indicates that the analyzed sample remains a complex mixture rather than a single isolated compound. The dominance of fluorescent zones at intermediate Rf values suggests a prevalence of semi-polar constituents, consistent with results obtained from phytochemical screening and solvent fractionation. This chromatographic pattern supports the pharmacological potential of the extract and provides a rational basis for bioassay-guided fractionation and further purification using preparative TLC or HPLC techniques.

CONCLUSION

The fruit of *Iris domestica* (L.) Goldblatt & Mabb exhibits satisfactory physicochemical quality, notable antibacterial activity, and significant hemostatic potential. The simplicia met pharmacopeial standards, indicating stability, purity, and suitability as a pharmaceutical raw material. Phytochemical analysis identified alkaloids, flavonoids, glycosides, saponins, and tannins, with the ethyl acetate fraction showing the richest bioactive composition, suggesting that phenolic compounds and alkaloids primarily contribute to its biological activities. The ethanolic extract demonstrated concentration-dependent antibacterial activity against Gram-positive and Gram-negative bacteria, producing inhibition zones ranging from approximately 12.7 mm against *Staphylococcus aureus*, 11.3 mm against *Staphylococcus epidermidis*, 10.5 mm against *Pseudomonas aeruginosa*, 10.6 mm against *Salmonella typhi*, and 9.7 mm against *Klebsiella pneumoniae* at the highest tested concentration. These findings indicate moderate to strong broad-spectrum effects. It also significantly reduced clotting and bleeding times compared to the negative control, confirming hemostatic activity likely mediated by tannins, supported by alkaloids and saponins that enhance coagulation processes. Overall, these findings provide experimental evidence that I. domestica fruit is a promising antibacterial and hemostatic agent, supporting its traditional use in wound management. Further studies on bioassay-guided isolation, mechanistic pathways, and safety evaluation are required to support its development as a standardized herbal pharmaceutical.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Education, Culture, Research and Technology, Indonesia for providing a grant through the dosen pemula research scheme (PD) N0. 124 LPPM USM-Indonesia/VI/2025 DPPM Program and to the Sari Mutiara Indonesia University for facilitating this research activity.

REFERENCES

Atanasov, A. G., Zotchev, S. B., & Dirsch, V. M. (2021). Natural products in drug discovery: Advanced strategies and opportunities. Nature Reviews Drug Discovery, 20, 200–216. https://doi.org/10.1038/s41573-020-00114-z

Amarowicz, R., Maryniak, A., and Shahidi, F. (2005). TLC Separation of Methylated-Epigallocatechin-3-Gallate. *Czech J. Food Science, 23*(1), 36-39.

Ayustaningwarno, F., Nugraha, R., & Pratama, A. (2024). A critical review of ginger's (Zingiber officinale) antioxidant and anti-inflammatory properties and immunomodulatory potential. *Frontiers in Nutrition, 11,* 1364836. https://doi.org/10.3389/fnut.2024.1364836

BPOM RI-The Indonesian Food and Drug Administration. (2019). *Guidelines for Good Manufacturing Practices for Traditional Medicines (CPOTB).* Jakarta: BPOM RI.

https://standarpangan.pom.go.id. Accessed on 20Th October 2025 **[*In Indonesian language*]**

BPOM RI- The Indonesian Food and Drug Administration. (2000). *General Standard Parameters for Medicinal Plant Extracts.* First Edition. Jakarta: Departemen Kesehatan RI. Pp 10-11. **[*In Indonesian language*]**

Badran, M. M., & Afouna, M. I. (2021). Topical phytopharmaceuticals: Formulation design and evaluation. *Journal of Pharmaceutical Sciences, 110*(8), 2450–2466. https://doi.org/10.1016/j.xphs.2021.04.012

Ballester, P., Cerdá, B., Arcusa, R., Marhuenda, J., Yamedjeu, K., & Zafrilla, P. (2022). Effect of ginger on inflammatory diseases. *Molecules, 27*(21), 7223. https://doi.org/10.3390/molecules27217223

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Bandyopadhyay, B., Munsi, A., Mollah, K. I., Debnath, B., & Bishal, A. (2025). Formulation and Evaluation of Polyherbal Pain Relief Gel for its Effect on Rheumatoid Arthritis. *Journal of Young Pharmacists, 17*(2), 336–343. https://doi.org/10.5530/jyp.20251472

Bonfils, J. P., Pinguet, F., Culine, S., & Sauvaire, Y. (2001). Cytotoxicity of iridals, triterpenoids from Iris on human tumor cell lines A2780 and K562. *Planta Medica, 67*(1), 79–81. https://doi.org/10.1055/s-2001-10625

Cheng, Y., Fu, J., Chen, L., Li, L. L., & Qu, J. (2019). Diterpenoid glycosides and monoterpenoid glycosides from the fruits of Xanthium. *Journal of Asian Natural Products Research, 21,* 207–216. https://doi.org/10.1080/10286020.2018.1526788

Demarque, D. P., Dusi, R. G., Sousa, F. D. M. de, Grossi, S. M., Silverio, M. R. S., Lopes, N. P., & Espíndola, L. S. (2020). Mass spectrometry-based metabolomics approach in the isolation of bioactive natural products. *Scientific Reports, 10*(1), 1051. https://doi.org/10.1038/s41598-020-58046-y

Ebrahimi, F., Torbati, M., Mahmoudi, J., & Valizadeh, H. (2020). Medicinal plants as potential hemostatic agents. *Journal of Pharmacy & Pharmaceutical Sciences, 23,* 10–23. https://doi.org/10.18433/jpps30446

Farag, S. F., Kimura, Y., Ito, H., Takayasu, J., Tokuda, H., & Hatano, T. (2009). New isoflavone glycosides from Iris spuria L. cultivated in Egypt. *Journal of Natural Medicines, 63,* 91–95. https://doi.org/10.1007/s11418-008-0291-7

Goldblatt, P., & Manning, J. (2005). *Plants of the World Online*. Royal Botanic Gardens, Kew. https://powo.science.kew.org. Accessed on 20Th October 2025

Guo, Y., Wang, M., Liu, Q., Liu, G., Wang, S., & Li, J. (2023a). Recent advances in the medical applications of hemostatic materials. *Theranostics, 13*(1), 161–196. https://doi.org/10.7150/thno.79639

Guo, Y.-P., Yi, P., Shi, Q.-Q., Yu, R.-R., Wang, J.-H., Li, C.-Y., & Wu, H.-Q. (2023b). Cytotoxic compounds from Belamcanda chinensis (L.) DC induce apoptosis in triple-negative breast cancer cells. *Molecules, 28*(12), 4715. https://doi.org/10.3390/molecules28124715

Harborne, J. B. (1984). *Phytochemical methods: A guide to modern techniques of plant analysis.* 2nd edition. Chapman and Hall. https://doi.org/10.1007/978-94-009-5570-7

Hayet, E., Maha, M., Samia, A., Mata, M., Gros, P., Raida, H., Ali, M.M., Mohamed, A.S., Gutmann, L., Mighri, Z. (2008). Antimicrobial, antioxidant, and antiviral activities of Retama raetam (Forssk.) Webb flowers growing in Tunisia. *World J. Microbiol. Biotechnol, 24*, 2933–2940, https://doi.org/10.1007/s11274-008-9835-y.

Khatib, S., Faraloni, C., & Bouissane, L. (2022). Exploring the use of Iris species: Antioxidant properties, phytochemistry, medicinal and industrial applications. *Antioxidants, 11*(3), 526. https://doi.org/10.3390/antiox11030526

Khanna, V.G., dan Kannabiran K. (2008). Antimicrobal Activity of SaponinFraction of the Leaves of *Gymna sylvestre* and *Eclipta prostrate. Worid J Microbiol Biotecnol.* 24, 2737 -2740.

Kumaraswamy, M.V., Kavitha, H.U., dan Satish, S. (2008). Antibakterial Evaluation and Phytochemical Analysis of Betula utilis D. Don Against Some HumanPathogenik Bakteria. *Advances in Biological Research, 2*(1-2), 21-25.

Li, J., Ni, G., Liu, Y., Wang, R., & Yu, D. (2022). Long-chain fatty acid acylated derivatives of isoflavone glycosides from the rhizomes of Iris domestica. *Phytochemistry, 193,* 112977. https://doi.org/10.1016/j.phytochem.2021.112977

Lewis, R. C., & Glueck, H. I. (1958). A micromethod for determining clotting time, using capillary blood and siliconized tubes. *The Journal of laboratory and clinical medicine*, *52*(2), 299–303.

Marcińczyk N, Gromotowicz-Popławska A, Tomczyk M and Chabielska E (2022) Tannins as Hemostasis Modulators. Front. *Pharmacol. 12,* 806891. https://doi.org/10.3389/fphar.2021.806891

Marpaung, J. K., Sitorus, P., & Suryanto, D. (2020). Fractionation and characterization of Iris domestica (L.) fruit ethanol extracts and antibacterial activities of each fraction. *Asian Journal of Pharmaceutical Research and Development, 8*(6), 46–52. https://doi.org/10.22270/ajprd.v8i6.814

Montazerian, H., Davoodi, E., Baidya, A., Baghdasarian, S., Sarikhani, E., Meyer, C. E., Haghniaz, R., Badv, M., Annabi, N., Khademhosseini, A., & Weiss, P. S. (2022). Engineered Hemostatic Biomaterials for Sealing Wounds. *Chemical Reviews*, *122*(15), 12864–12903. https://doi.org/10.1021/acs.chemrev.1c01015

Samavati, S. S., Kashanian, S., Derakhshankhah, H., Rabiei, M., Sajadimajd, S., Fakhri, S., & Rashidi, Z. (2025). Accelerated wound healing through tannin-rich Quercus brantii extract: Concentration-dependent efficacy and mechanistic insights. *Scientific Reports, 15,* 29004. https://doi.org/10.1038/s41598-025-13832-4

Schmid, R., Petras, D., et al. (2021). Ion identity molecular networking for mass spectrometry-based metabolomics in the GNPS environment. *Nature Communications, 12*(1), 3462. https://doi.org/10.1038/s41467-021-23953-9

Shaikh, J. R., & Patil, M.K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies, 8*(2), 603–608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Siafaka, P. I., Miliotou, A. N., Okur, M. E., Karaotmarlı Güven, G., Karantas, I. D., & Üstündağ Okur, N. (2025). Nanoformulations Loaded with Phytochemicals for Combating Wound Infections and Promoting Wound Healing: Current Applications and Innovations. *Applied Sciences*, *15*(10), 5413. https://doi.org/10.3390/app15105413

Theisen, L. L., Erdelmeier, C. A. J., Spoden, G. A., Boukhallouk, F., Sausy, A., Florin, L., & Muller, C. P. (2014). Tannins from Hamamelis virginiana Bark Extract: Characterization and Improvement of the Antiviral Efficacy against Influenza A Virus and Human Papillomavirus. *PLoS ONE*, *9*(1), 1-14. https://doi.org/10.1371/journal.pone.0088062

World Health Organization. (2006). *WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues*. Geneva: WHO. https://apps.who.int/iris/handle/10665/43510

How To Cite This Article, with *APA style* :

Marpaung, J. K., Tampubolon, M. I., & Adiansyah, A. (2026). Phytochemical Profiling and Hemostatic Potential of *Iris domestica* Fruit Extract for Wound Bleeding Control. *Jurnal Pembelajaran dan Biologi Nukleus*, *12*(1), 213-227. https://doi.org/10.36987/jpbn.v12i1.9059

Conflict of interest : The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions : All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was submited by [**Jon Kenedy Marpaung**]. All authors contributed on previous version and revisions process of the manuscript. All authors read and approved the final manuscript.