

LC-MS-Based Phytochemical Profiling of *Priyangvadi choorna* and its Correlation with Hemostatic Activity

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ABSTRACT

Background: *Priyangvadi Churna* is a traditional indigenous *Ayurvedic* Polyherbal formulation explained in the treatment of bleeding disorders (Raktapitta) by *Yogaratanakar*. In spite of its renowned efficacy in standard *Ayurvedic* textbooks, there is a deficiency of phytochemical representation to back its hemostatic qualities through existing analytical procedures. **Objectives:** To identify and illustrate bioactive elements of *Priyangvadi Churna* using LC-MS and their applicability to hemostatic activity. **Materials and Methods:** Methanolic extracts of *Priyangvadi Churna* were tested with a Waters 1525 μ Binary Pump LC system and a Waters Xevo G2-XS QT of mass spectrometer. **Results:** A total of 454 distinct phyto-constituents were documented, 223 are unknown. These includes flavonoids, tannins, Alkaloids, etc, were consistent with *Ayurvedic* therapeutic actions like Raktasthambhaka (blood-stopping) and Shothahara (anti-inflammatory). **Conclusion:** The LC-MS profile of *Priyangvadi Churna* justifies its classical use as a hemostatic agent. Identified bioactive compounds show pharmacological potential for clotting enhancement and vascular integrity.

Keywords: *Priyangvadi Churna*, LC-MS, Hemostatic, Phytochemical profiling, *Ayurveda*, Flavonoids, Tannins.

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INTRODUCTION

Bleeding is one of the important global health concerns. The commonest causes are injuries, surgeries, obstetrics, and pathological disorders. Traumatic bleeding is the leading cause of preventable death, contributing to nearly 40% of global mortality in early trauma (Watson *et al.*, 2022). Non-cardiac perioperative bleeding accounts for approximately 11.6% of cases within 30 days following surgery (Alderliesten *et al.*, 2024). Globally, bleeding significantly increases the duration of hospital stay and healthcare expenditure and adversely affects clinical outcomes (Leff *et al.*, 2020).

Despite advances in hemostatic management, both local and systemic agents are currently in use with notable limitations. Local hemostatic agents may interfere with wound healing and increase the risk of infection (Achneck *et al.*, 2010; Spotnitz and Burks, 2012). Systemic agents, such as antifibrinolytics, are associated with adverse effects including thromboembolic events, hypersensitivity reactions, and renal complications, particularly

in susceptible individuals (Ker *et al.*, 2012; Levi and Hunt, 2015). These challenges emphasize the need for safer, targeted, and biocompatible alternatives, including plant-derived and polyherbal hemostatic formulations.

In *Ayurveda*, Raktasthambhanopāyas (haemostatic measures) are described in the management of Raktātīpravṛtti (excessive bleeding) and include therapeutic principles such as Sandhāna, Skandhana, Pācana, and Dāhana (Sharma, 2001). Among the formulations employed under Sandhāna therapy, *Priyangvadi Choorna* is a traditional polyherbal preparation indicated for various bleeding disorders such as epistaxis, oral bleeding, anorectal bleeding, vaginal bleeding, penile bleeding, and wounds caused by sharp instruments, as documented in *Yogaratanakara* (Shastri, 2010).

The formulation is reported to possess hemostatic, anti-inflammatory, antimicrobial, and wound-healing properties (Sharma *et al.*, 2001; Shastri, 2010). *Priyangvadi Choorna* is traditionally administered using classical *Ayurvedic* methods, often with *Vasa swarasa*. The present work represents the first application of Liquid Chromatography-Mass Spectrometry (LC-MS) for chemical profiling of this formulation. LC-MS analysis revealed the presence of multiple phytoconstituents known for their anti-inflammatory, antioxidant, and cytoprotective activities.



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MATERIALS AND METHODS

Priyangvadi Choorna was obtained from GMP Certified Ayurveda pharmacy Khasbag, Belgavi, mentioned in Table 1 (Government of India, Department of AYUSH, 2003) and analysed using LC-MS by standard protocols.

Study conduction

Our study was conducted in Biocyte Research and Development Pvt. Ltd., Sangli, Maharashtra.

Simple Preparation

After purchasing *Priyangvadi Choornam* in powdered form, 100 mg of the sample was precisely weighed and diluted in 100 mL of methanol. To ensure full extraction of phytochemical contents, the solution underwent sonication for 20 min. After sonication, the solution was filtered to produce an extract clear enough for LC-MS analysis. Analysis of Liquid Chromatography-Mass Spectrometry (LC-MS) was conducted utilizing a Waters Xevo G2-XS QT mass spectrometer alongside a Waters 1525 μ Binary Pump liquid chromatography system. Chromatographic separation was carried out using an Accucore C18 column (50 mm \times 4.6 mm, 5 μ m particle size; ThermoScientific). The mobile phases consisted of Acetonitrile (solvent B) and 0.1% formic acid in water (solvent A). The gradient elution schedule used was: 95% A and 5% B from 0 to 1 min, transitioning to 50% A and 50% B by 8 min, moving to 5% A and 95% B by 12 min, maintained until 17 min, then returning to 95% A and 5% B by 18 min, which remained until 20 min. Throughout the run, the injection volume stayed at 10 μ L, and the flow rate was held steady at 0.5 mL/min.

Mass Spectrometry Parameters

The mass spectrometer functioned in positive and negative Electrospray Ionization (ESI) modes to detect each component present. The collision energy was configured to 20 V, and the capillary voltage was adjusted to 3.0 kV. The collision energy could range from 30 to 90 V. The temperatures for the source and desolvation were held constant at 150°C and 450°C, respectively. The gas flow rate for desolvation was 800 L/h, whereas the cone gas flow rate stood at 50 L/h. Data Gathering Information was obtained using MassLynx software (version 4.1). For analyzing the chemical composition of the plant sample, both positive and negative ionization modes can be used to produce Total Ion Chromatograms (TIC) and Base Peak Intensity (BPI) chromatograms.

Ethical Statement

Not applicable, as this study involved an *in vitro* phytochemical analysis of a commercial product. No human or animal subjects were used.

Statistical Analysis

Because the study concentrated on analytical profiling without assessing differences between groups, inferential statistical approaches were not applicable.

RESULTS

The LC-MS analysis of *Priyangvadi Choorna* was performed in both negative and positive ionization modes. Figure 1 shows the negative mode LC-TIC and BPI chromatograms, while Figure 2 depicts the positive mode LC-TIC and BPI chromatograms. The analysis revealed the presence of multiple bioactive phytochemicals, including flavonoids (Tectochrysin, Kaempferol, Liquiritigenin, Hesperetin, Ononin), alkaloids (Sanguinarine), phenolic acids (Ellagic acid, 1,3-Benzenedicarboxylic acid), and other compounds such as Thalidomide and 4-Methylumbelliferone.

Several of these constituents, including Ellagic acid, Hesperetin, Sanguinarine, and Tectochrysin, have been reported to modulate platelet activation, thrombus formation, and vascular health. Ellagic acid acts as a haemostatic agent by activating Factor XII in the intrinsic coagulation pathway. Sanguinarine modulates the GPVI signaling pathway and regulates thromboxane, thereby controlling platelet activation. Hesperetin and Tectochrysin inhibit platelet aggregation and inflammation, helping maintain endothelial stability and preventing thrombosis. Additionally, 4-Methylumbelliferone (4-MU) and Biochanin A exert antioxidant and anti-inflammatory effects, contributing to vascular protection and maintaining haemostatic balance.

These findings support the traditional use of *Priyangvadi Choorna* in wound healing and bleeding management. Table 2 summarizes the phytochemicals detected in the acetone-extracted *Priyangvadi Choorna* by LC-MS, highlighting their potential role in modulating platelet function, stabilizing the endothelium, and maintaining haemostatic equilibrium.

DISCUSSION

LC-MS (liquid chromatography-mass spectrometry) analysis identified several compounds with potential haemostatic properties, including flavonoids, alkaloids, and phenolic acids such as tectochrysin, hesperetin, kaempferol, biochanin A, 4-methylumbelliferone, ononin, columbianetin, and ellagic acid. Ellagic acid enhances coagulation by activating Hageman factor (factor XII) in the intrinsic pathway, resulting in increased serine protease activity. It is a naturally occurring polyphenolic compound and is also known for its antiproliferative, wound-healing, and antioxidant properties (Gopalakrishnan *et al.*, 2014; Umesalma and Sudhandiran, 2011). Sanguinarine, an alkaloid belonging to the benzophenanthridine class, exhibits anti-inflammatory, antioxidant, and anticancer activities. It attenuates collagen-induced platelet activation and thrombus

formation by modulating the GPVI pathway, reducing intracellular calcium mobilization and integrin $\alpha\text{IIb}\beta\text{3}$ -mediated outside-in signaling (Shu *et al.*, 2021). Biochanin A, an isoflavone, enhances vasorelaxation in coronary arteries and may indirectly support haemostasis by maintaining vascular tone. It also exhibits neuroprotective, anti-inflammatory, anticancer, and antioxidant properties through modulation of NF- κB , AP-1, and JAK/STAT signaling pathways (Feng and Lai, 2023; Sobotková *et al.*, 2009). Tectochrysin, a methylated flavonoid, demonstrates antithrombotic activity by inhibiting NF- κB signaling, thereby reducing platelet aggregation and pro-inflammatory cytokine expression. Additionally, it exhibits anti-inflammatory, antibacterial, hepatoprotective, neuroprotective, and antioxidant effects (Lee *et al.*, 2003; Lu *et al.*, 2020). Liquiritigenin reduces oxidative stress and extracellular matrix accumulation under hyperglycaemic conditions by suppressing NF- κB and NLRP3 inflammasome activation, thereby promoting wound healing (Zhu

et al., 2018). 4-Methylumbelliferone exhibits anti-inflammatory effects by regulating MAPK, SYK, and NF- κB signaling pathways. Although it does not directly influence coagulation, it helps maintain endothelial integrity, which is essential for vascular homeostasis (Wang *et al.*, 2022). Kaempferol prevents platelet activation and thrombus formation while offering vascular protection. It also possesses anti-inflammatory, antioxidant, and anticancer activities (Lee *et al.*, 2018; Liu *et al.*, 2020). Ononin reduces inflammatory cytokine production by downregulating inflammatory mediator mRNA expression via inhibition of the NOD1/RIP2/NF- κB signaling pathway (Yu *et al.*, 2023). Columbianetin exhibits significant anti-inflammatory and antioxidant effects in immune cells (Lu *et al.*, 2018). Hesperetin, a bioflavonoid, exhibits antioxidant, anti-inflammatory, antihypertensive, antiatherogenic, and anticancer properties. It reduces oxidative stress, DNA damage, and cellular apoptosis by modulating p38 MAPK, NF- κB , and caspase-3 signaling

Table 1: List of Ingredients Used in the Formulation of *Priyangvadi Choorna*.

Drug	Latin name	Family	Part used
<i>Priyangu</i>	<i>Callicarpa macrophylla</i>	Verbanace	flower
<i>Lodra</i>	<i>Symplocos recemosa</i>	symplococeae	Root bark
<i>Vasa</i>	<i>Adathoda vasica</i>	acanthaceae	leaves
<i>Shodhitha Sphatika</i>	<i>Pottash alum</i>	-	Powdered form of whole mineral drug
<i>Rasanjana</i>	<i>Berberis aristata</i>	Berberidaceae	Rhizome

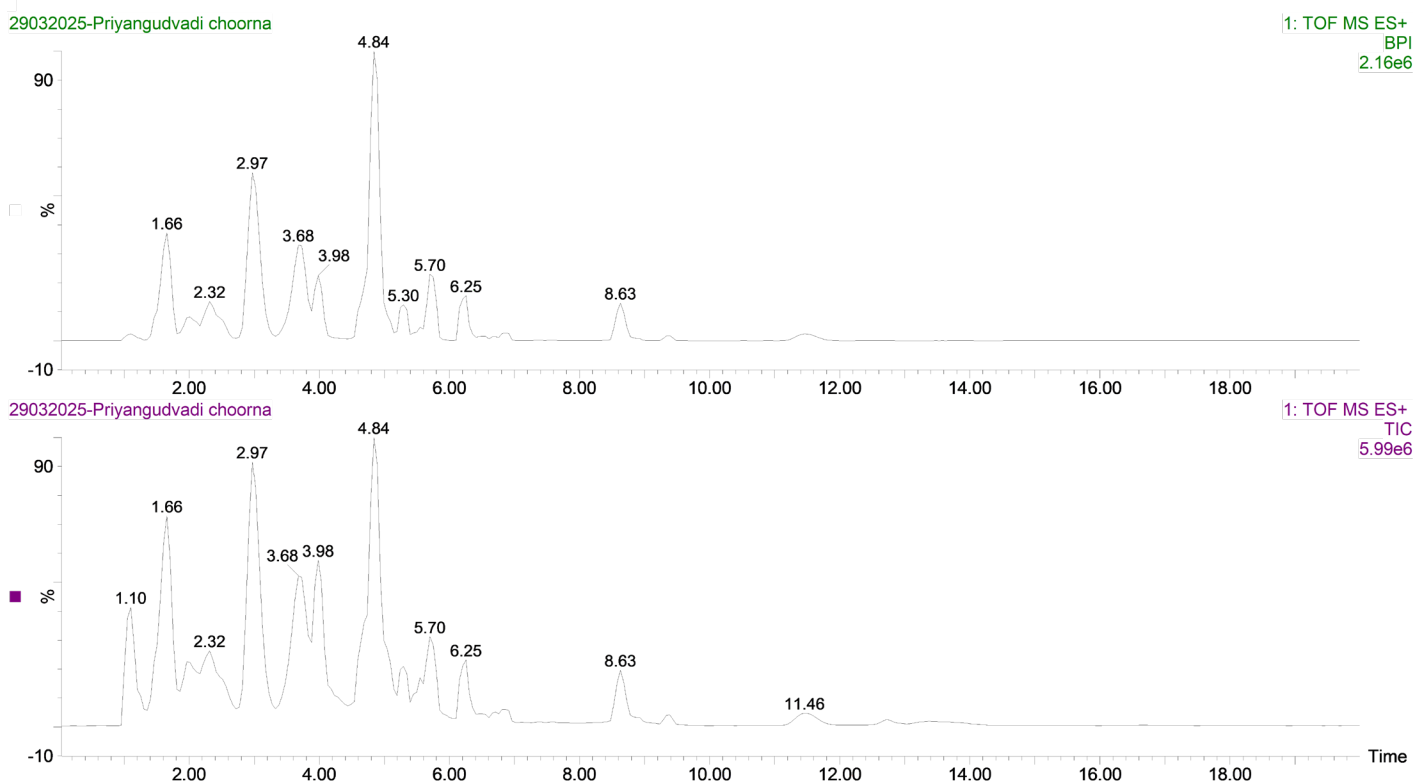


Figure 1: Negative mode LC-TIC and BPI Chromatograms.

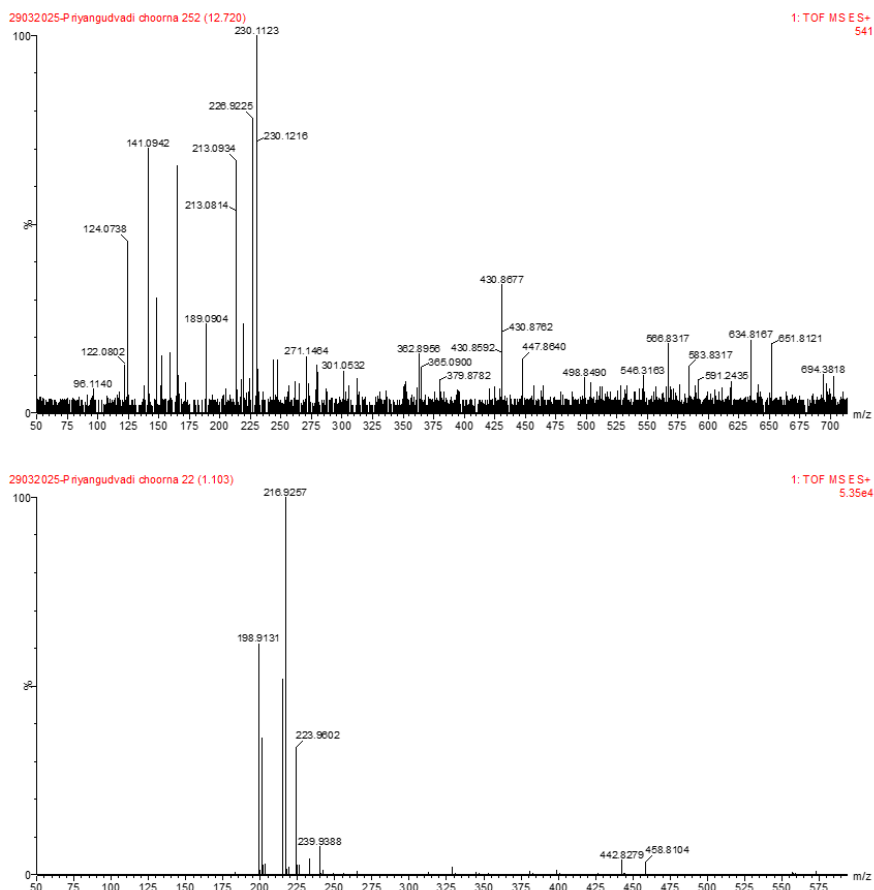


Figure 2: Positive mode LC - TIC and BPI Chromatograms.

Table 2: Phytochemicals detected in the acetone-extracted *Priyngvadi choorna* using LC-MS.

Compound Name	RT (min)	Precursor m/z	Area	Ion Mode
Tectochrysin	5.247	267.06332	9053673	Negative
Constrictic acid	4.843	447.05624	7490154	Negative
Liquiritigenin	6.507	279.05902	1190501	Positive
4-methylumbelliferone	1.052	198.91307	934112	Positive
Sanguinarine	1.204	332.09515	348657	Positive
Liquiritigenin	8.225	279.0556	169946	Positive
Hesperetin	4.84	303.19116	152093	Positive
5-Methoxytryptamine	5.649	191.1165	93424	Positive
Phenazone	4.537	189.10165	91635	Positive
Biochanin A	7.366	285.17105	87158	Positive
Ononin	4.487	453.12027	78514	Positive
Thalidomide	5.245	259.07053	47245	Positive
1,3-Benzenedicarboxylic acid	1.205	165.01917	45886	Negative
Kaempferol-7-O-glucoside	5.247	447.09521	42224	Negative
L-Tryptophan	5.8	205.09718	33407	Positive
Coumarin base + 1O, 1MeO	1.205	191.034	9,406,799	Negative
Ellagic acid	4.840	303.013	57,638,887	Positive
Laxapur	4.287	239.035	24,923,317	Negative
Khellin	6.305	261.081	1,571,245	Positive

Table 3: Pharmacological Activity of Compounds Found in LCMS of *Priyngvadi Choorna*.

Compound	Molecule formula	Pharmacological action
Tectochrysin	$C_{16}H_{12}O_4$	Anti-inflammatory, Anti-cancer, Anti-bacterial, Hepatoprotective and neuroprotective effects Antioxidant.
Constrictic acid	$C_{19}H_{14}O_{10}$	Antimicrobial Antioxidant 38.
Liquiritigenin	$C_{15}H_{12}O_4$	Anti-inflammatory, Anti-hyperlipidaemia, anti-oxidative.
4-methylumbelliferone	$C_{10}H_8O_3$	Inflammatory responses Haemostatic function Maintaining vascular integrity.
Sanguinarine	$C_{20}H_{14}NO_4$	Anti-inflammatory, antioxidant Antitumor. Platelet activation.
Liquiritigenin	$C_{15}H_{12}O_4$	Anti-inflammatory, Anti-hyperlipidaemia, Anti-oxidative.
Hesperetin	$C_{16}H_{14}O_6$	Antiplatelet agent.
5-Methoxytryptamine	$C_{11}H_{14}N_2O$	Antioxidant Anti-inflammatory Neuroprotective.
Phenazone	$C_{11}H_{12}N_2O$	Analgesic Anti-inflammatory.
Biochanin A	$C_{16}H_{12}O_5$	Anti-inflammatory, anti-oxidant, Anti-cancer neuroprotective.
Ononin	$C_{22}H_{22}O_9$	Anti-inflammatory.
Thalidomide	$C_{13}H_{10}N_2O_4$	Procoagulant activity Anti-inflammatory.
1,3-Benzenedicarboxylic acid	$C_8H_6O_4$	Antiinflammatory Anticancer / Antitumor Activity.
Kaempferol-7-O-glucoside	$C_{21}H_{20}O_{11}$	Anti-inflammatory, Anti-cancer, Antibacterial,
L-Tryptophan	$C_{11}H_{12}N_2O_2$	Antioxidant 40.
Coumarin base + 1O, 1MeO		Improved venous circulation, anti-neoplastic. Anticancer agents Antibacterial activities, antioxidant.
Ellagic acid	$C_{14}H_6O_8$	Anti- inflammatory Anti-oxidant Anticancer.
Laxapur	$C_{14}H_8O_4$	Antioxidant Anti -inflammatory.

Compound	Molecule formula	Pharmacological action
Khellin	C ₁₄ H ₁₂ O ₅	Anti-inflammatory antioxidant, Antimicrobial activities, Vaso dialator.

pathways, thereby reducing doxorubicin-induced cardiotoxicity (Rehman *et al.*, 2024). Thalidomide exhibits procoagulant effects under specific conditions by increasing tissue factor expression and thrombin generation in endothelial cells and also possesses anti-inflammatory and anti-angiogenic properties (Akter *et al.*, 2022; Chen *et al.*, 2009; Qiao *et al.*, 2017). Isophthalic acid (1,3-benzenedicarboxylic acid) exhibits antibacterial and antifungal activities (Hamsalakshmi *et al.*, 2021). Coumarins are naturally occurring compounds with diverse biological properties, including antibacterial, anti-inflammatory, and anticancer activities (Garrard, 2014; Lake, 1999; Önder, 2020). Laxapur, a flavonoid compound, acts as a potent free-radical scavenger and exerts anti-inflammatory effects by inhibiting NF-κB signaling and reducing pro-inflammatory mediators such as iNOS and COX-2 (Egbuna *et al.*, 2020; Lin *et al.*, 2009). Khellin is primarily known for its vasodilatory and smooth muscle relaxant properties (National Center for Biotechnology Information [NCBI], 2025a). Constrictic acid, a lichen-derived depsidone, exhibits antimicrobial and antioxidant activities (NCBI, 2025b). Antipyrine, a pyrazolone derivative, exhibits analgesic, antipyretic, anti-inflammatory, and platelet-inhibitory effects through inhibition of cyclooxygenase-mediated prostaglandin synthesis (NCBI, 2025c). Tryptophan, an essential amino acid, serves as a precursor for bioactive molecules such as melatonin and kynurenine, contributing to antioxidant and immunomodulatory effects (Ghazaghi *et al.*, 2024). 5-Methoxytryptamine, a serotonin derivative, exhibits free-radical scavenging activity and suppresses inflammation via cytokine modulation (NCBI, 2025d; Tan *et al.*, 1993).

CONCLUSION

A detailed phytochemical investigation of *Priyangvadi Choorna* through LC-MS analysis revealed the presence of 454 compounds, among them 223 are unknown molecules which may exhibit coagulative properties. The known compounds, particularly those associated with haemostasis, inflammatory control, and wound healing. Principal ingredients, including Ellagic acid, Sanguinarine, Hesperetin, Tectochrysin, and Liquiritigenin, indicated established functions in augmenting coagulation pathways and maintaining endothelial integrity. No sufficient study has been conducted for particularly for haemostatic activity in many compounds found in *Priyangvadi Choorna*. These results illustrate that the traditional usage of *Priyangvadi Choorna* is a treatment for bleeding disorders, inflammatory diseases, and wound healing issues. The findings further underscore its promise

as a natural, plant-derived treatment for the development of innovative haemostatic medicines.

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ABBREVIATIONS

LC-MS: Liquid Chromatography-Mass Spectrometry; **ESI:** Electrospray Ionization; **TIC:** Total Ion Chromatogram; **BPI:** Base Peak Intensity; **RT:** Retention Time; **QToF:** Quadrupole Time-of-Flight; **4-MU:** 4-Methylumbelliferone; **NF-κB:** Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells; **MAPK:** Mitogen-Activated Protein Kinase; **NLRP3:** NOD-like Receptor Family Pyrin Domain Containing 3; **JAK/STAT:** Janus Kinase/Signal Transducer and Activator of Transcription; **PI3K:** Phosphoinositide 3-Kinase; **SYK:** Spleen Tyrosine Kinase; **GPVI:** Glycoprotein VI; **Factor XII:** Hageman.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Dr. Adithya Anil conceived and designed the study, conducted the experimental work, performed data acquisition and analysis, and prepared the original draft of the manuscript. Dr. Santosh Y. Mudakappagol contributed to study supervision, methodological guidance, and critical revision of the manuscript for important intellectual content. Dr. Raj Joshi assisted in data interpretation, literature review, and manuscript editing and review. All authors read and approved the final version of the manuscript.

SUMMARY

This study explores the phytochemical composition and hemostatic relevance of *Priyangvadi Churna*, a classical Ayurvedic polyherbal formulation traditionally used in the management of bleeding disorders (Raktapitta). Using Liquid Chromatography-Mass Spectrometry (LC-MS), methanolic extracts of the formulation were analyzed to characterize its bioactive constituents. The analysis identified 454 phytochemicals, including flavonoids, tannins, alkaloids, and phenolic compounds, of which 223 remain unidentified. Several identified constituents are known to possess hemostatic, anti-inflammatory, antioxidant,

and vascular-protective properties, correlating with classical Ayurvedic actions such as Raktasthambhaka and Shothahara. The findings provide analytical evidence supporting the traditional use of *Priyngvadi Churna* as a natural hemostatic agent and highlight its potential as a source of plant-derived compounds for developing safer and effective hemostatic therapies.

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