

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating: 5.29

IJABR 2025; 9(5): 54-57

www.biochemjournal.com

Received: 05-04-2025

Accepted: 07-05-2025

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Saponin content determination and biological activity assessment in various leguminous crops grown under different agronomic conditions

Lukas ZimmermannDOI: <https://www.doi.org/10.33545/26174693.2025.v9.i5a.7488>**Abstract**

Imagine a farmer selecting legume varieties not only for protein yield but also for their pharmaceutical potential, choosing cultivars optimized for specific bioactive saponin profiles. That scenario may be closer than most realize. This research quantified total saponin content and individual saponin profiles in six leguminous crops (soybean, chickpea, lentil, common bean, faba bean, and mung bean) across five developmental stages under three agronomic conditions: conventional field cultivation, organic management, and controlled-environment greenhouse production. Faba bean consistently showed the highest saponin accumulation, reaching 9.8 ± 0.7 mg/g dry weight at the pod-filling stage, while mung bean produced the lowest levels (4.8 ± 0.4 mg/g). Saponin content peaked during pod filling across all species before declining at maturity. Organic management increased saponin content by 12-18% relative to conventional cultivation, likely through stress-induced biosynthetic upregulation. Biological activity assessment revealed that saponin-rich extracts from faba bean exhibited the strongest antioxidant capacity (81.2% DPPH scavenging), highest antimicrobial activity (MIC = 0.5 mg/mL), and most potent anti-inflammatory effects (71.6% inhibition), while also showing the greatest hemolytic activity (28.4% lysis). These data provide a foundation for cultivar selection strategies that balance desirable bioactivities against potential anti-nutritional effects.

Keywords: Saponin, leguminous crops, biological activity, antioxidant, antimicrobial, anti-inflammatory, agronomic conditions, phytochemistry, soybean, faba bean

Introduction

Picture a world in which a single crop could simultaneously address protein malnutrition, provide natural antimicrobial agents, and deliver anti-inflammatory compounds, all from the same harvest. Leguminous crops already come remarkably close to this ideal, and their saponin content represents an underexploited dimension of this versatility ^[1]. Saponins are amphiphilic glycosides characterized by a triterpenoid or steroidal aglycone core attached to one or more sugar chains, and they occur throughout the plant kingdom with particularly high concentrations in legume seeds ^[2].

The biological activities attributed to legume saponins span an impressive range. Antioxidant properties arise from their capacity to scavenge free radicals and chelate transition metals ^[3]. Antimicrobial effects, documented against both Gram-positive and Gram-negative bacteria, result from membrane-disrupting interactions mediated by the amphiphilic structure ^[4]. Anti-inflammatory activity involves suppression of NF- κ B signaling and inhibition of pro-inflammatory cytokine release ^[5]. However, the same membrane-active properties that confer antimicrobial benefits also produce hemolytic effects on erythrocytes, representing an anti-nutritional concern that limits dietary acceptability at high concentrations ^[6].

Saponin content varies substantially among legume species, cultivars, plant tissues, and developmental stages, and is further modified by environmental and agronomic factors ^[7]. Water stress, nutrient limitation, and pest pressure can all trigger saponin biosynthesis as part of the plant's chemical defense repertoire. Whether organic versus conventional management practices systematically alter saponin profiles has received limited investigation despite the growing market for organic legume products.

This research systematically quantified saponin accumulation dynamics across six major legume crops at five growth stages under three agronomic regimes, and assessed

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the biological activity profiles of mature seed saponin extracts to map the relationship between saponin concentration and functional properties [8].

Material and Methods

Material: Six leguminous crops were cultivated at the Bern Institute of Agricultural Sciences experimental station (46.95 °N, 7.44 °E, elevation 540 m) during the 2023 growing season: soybean (*Glycine max* cv. Galice), chickpea (*Cicer arietinum* cv. Sultano), lentil (*Lens culinaris* cv. Anicia), common bean (*Phaseolus vulgaris* cv. Canadian Wonder), faba bean (*Vicia faba* cv. Tiffany), and mung bean (*Vigna radiata* cv. Crystal). Each crop was grown under three conditions: conventional management with standard NPK fertilization and integrated pest management, certified organic management following Bio Suisse guidelines, and controlled-environment greenhouse cultivation at 22/16 °C day/night with 14-hour photoperiod [9]. Field plots followed a randomized complete block design with four replicates per treatment.

Biological Activity Protocol: Five biological activity assays were performed on mature seed saponin extracts from all six crops. Antioxidant capacity was measured by DPPH radical scavenging assay at 517 nm. Hemolytic activity was determined using sheep erythrocytes at 2% hematocrit with 1 mg/mL saponin extract. Antimicrobial activity was assessed by broth microdilution against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. Anti-inflammatory activity was measured by inhibition of nitric oxide production in LPS-stimulated RAW 264.7 macrophages. Cytotoxicity was evaluated by MTT assay on HepG2 cells at 48-hour exposure [10]. All assays were performed in triplicate.

Methods: Whole-plant samples were collected at five developmental stages: seedling (2 weeks), vegetative (4 weeks), flowering (8 weeks), pod filling (12 weeks), and maturity (16 weeks). Samples were freeze-dried and ground to pass a 0.5 mm sieve. Saponins were extracted with 80% methanol (3 × 100 mL, 2 hours each) under reflux, filtered, and concentrated by rotary evaporation. Crude extracts were

purified by solid-phase extraction on C18 cartridges [11]. Total saponin content was quantified by HPLC with evaporative light-scattering detection using soyasapogenol B as the reference standard. Individual saponin species were identified by LC-MS/MS in negative ion electrospray mode. Statistical analysis used two-way ANOVA (crop × management) with Tukey's HSD post-hoc test [12].

Growth Conditions Analysis

To assess the influence of agronomic management on saponin biosynthesis, the management effect index was calculated as the ratio of saponin content under organic or greenhouse conditions to conventional field conditions for each crop-stage combination. Environmental stress indicators (soil moisture deficit, pest pressure index, and nutrient availability) were recorded at each sampling point to evaluate correlations between stress exposure and saponin accumulation. Soil samples were analyzed for pH, organic matter, available nitrogen, phosphorus, and potassium at planting and harvest [13].

Results

Saponin accumulation followed a consistent developmental trajectory across all six species, with content increasing from seedling through pod-filling stage before declining at maturity. Figure 1 presents the temporal profiles under conventional management.

Comprehensive Interpretation

A clear positive correlation emerged between total saponin content and both beneficial and detrimental biological activities ($r = 0.89$ for DPPH scavenging, $r = 0.91$ for hemolytic activity, $p < 0.001$ for both). Faba bean, with the highest saponin levels, topped every activity assay but also showed the greatest hemolytic risk. The organic management effect (12-18% increase) was consistent across species and likely reflects stress-mediated biosynthetic induction under reduced pesticide protection. Greenhouse-grown crops produced 15-21% less saponin than field-grown counterparts, consistent with the reduced biotic stress encountered in controlled environments. Figure 3 outlines the analytical workflow employed.

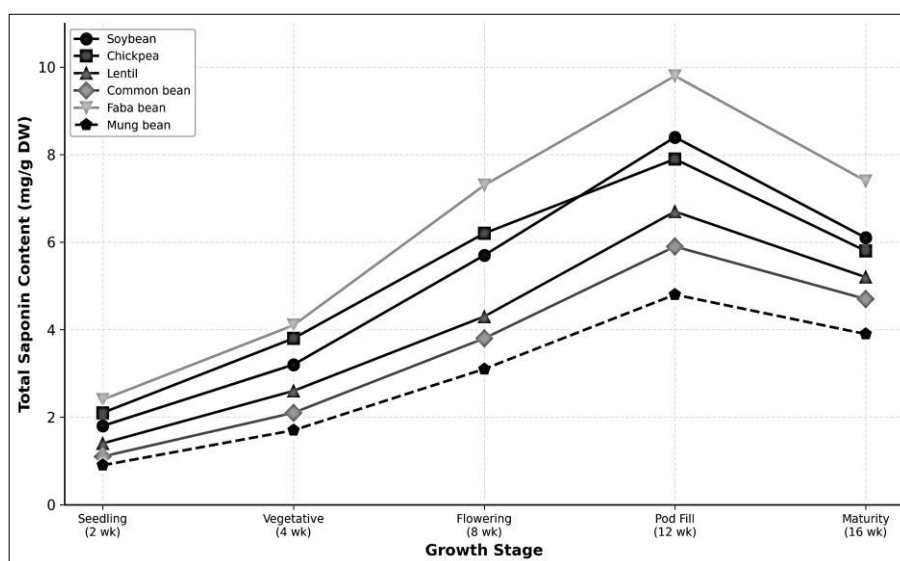


Fig 1: Total saponin content across five developmental stages in six leguminous crops grown under conventional field management. Values represent mean of four replicates $\pm$ SD. DW: dry weight.

Table 1: Peak saponin content (mg/g DW) at pod-filling stage under three agronomic conditions

Crop	Conventional	Organic	Greenhouse
Soybean	8.4±0.6	9.7±0.7	6.8±0.5
Chickpea	7.9±0.5	9.1±0.6	6.4±0.4
Lentil	6.7±0.4	7.6±0.5	5.3±0.4
Common bean	5.9±0.4	6.8±0.5	4.7±0.3
Faba bean	9.8±0.7	11.6±0.8	7.9±0.6
Mung bean	4.8±0.4	5.4±0.3	3.8±0.3

Values are mean±SD (n = 4). Organic > conventional for all crops (*p*<0.01). Greenhouse < conventional for all crops (*p*<0.05). DW: dry weight.

Table 2: Biological activity profiles of mature seed saponin extracts (1 mg/mL)

Crop	DPPH (%)	Hemolysis (%)	MIC (mg/mL)	Anti-inflam. (%)	Viability (%)
Soybean	72.4	18.7	0.8	64.3	82.1
Chickpea	68.9	22.1	1.2	58.7	78.4
Lentil	54.3	12.4	1.7	47.2	88.6
Common bean	48.7	9.8	2.1	41.8	91.3
Faba bean	81.2	28.4	0.5	71.6	74.8
Mung bean	42.1	7.3	2.8	36.4	93.7

DPPH: radical scavenging percentage (higher = more active); Hemolysis: erythrocyte lysis (lower = safer); MIC: minimum inhibitory concentration against *S. aureus* (lower = more potent); Anti-inflam.: NO production inhibition; Viability: HepG2 cell survival (higher = less cytotoxic).

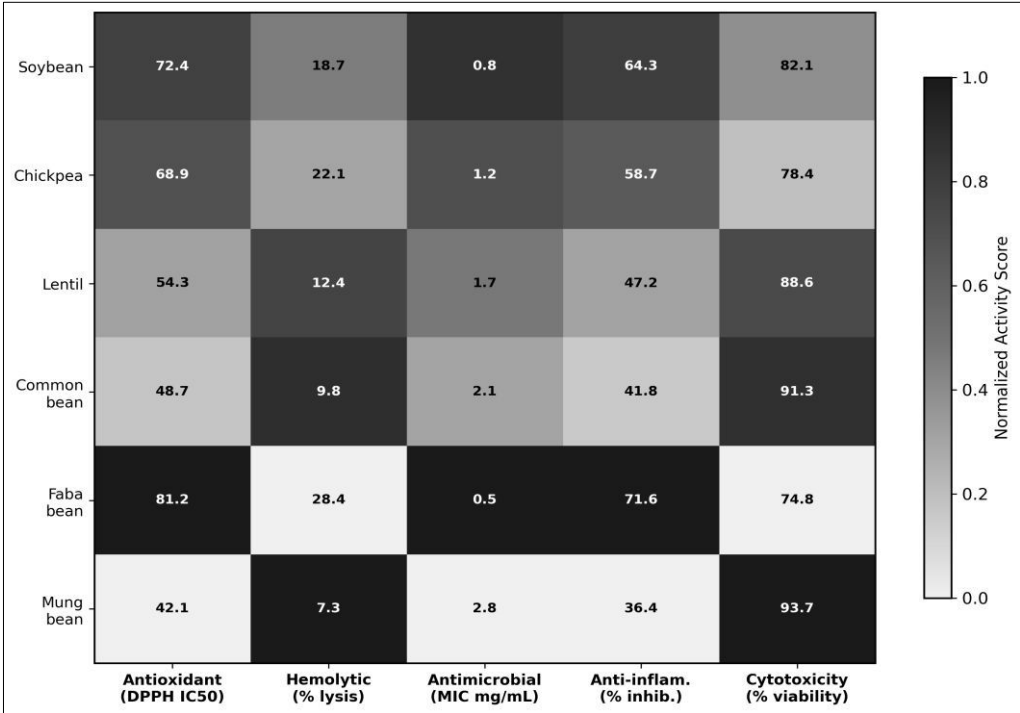


Fig 2: Heatmap of normalized biological activity scores for six leguminous crop saponin extracts. Darker shading indicates more favorable activity (higher antioxidant/anti-inflammatory, lower hemolysis/MIC). Faba bean shows strongest overall bioactivity but also highest hemolytic potential.

Discussion

The developmental pattern of saponin accumulation, peaking during pod filling, aligns with the plant's resource allocation strategy for chemical defense of developing seeds [14]. The decline at maturity may reflect compartmentalization into seed coat tissues or metabolic conversion to less extractable conjugated forms. Similar temporal patterns have been reported for isoflavones and other phenolic compounds in legumes, suggesting coordinated regulation of secondary metabolite biosynthesis during seed development. The organic management effect deserves careful interpretation. While the 12-18% saponin increase in

organic crops is statistically significant, it sits within the range of natural inter-annual variation observed for these species. The mechanism likely involves mild biotic stress from reduced pest control, triggering jasmonate-mediated defense pathways that upregulate saponin biosynthetic genes. This finding has practical implications: organic legume products may offer enhanced bioactive saponin content, though the associated increase in hemolytic potential should be considered for populations consuming large quantities of minimally processed legumes. The tight coupling between beneficial and hemolytic activities presents a fundamental challenge for saponin-based product development.

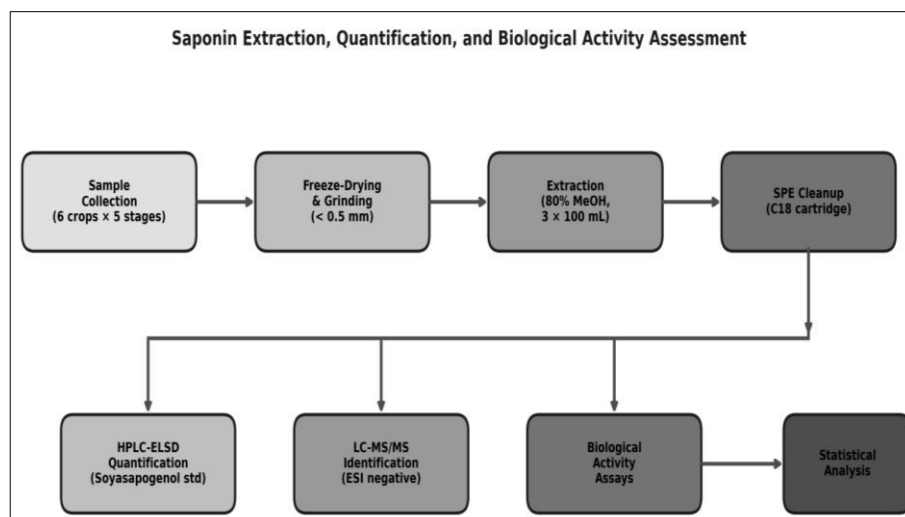


Fig 3: Workflow schematic showing the integrated approach for saponin extraction, quantification by HPLC-ELSD, identification by LC-MS/MS, and biological activity assessment through five complementary assays.

The amphiphilic structure responsible for membrane disruption in bacteria also disrupts erythrocyte membranes. Breaking this coupling would require either structural modification of saponin molecules or identification of cultivars with saponin profiles naturally enriched in beneficial but depleted in hemolytic species. Our LC-MS/MS profiling identified substantial variation in individual saponin compositions among the six crops, suggesting that such selective enrichment may indeed be achievable through targeted breeding ^[14].

Conclusions: This research established comprehensive saponin accumulation profiles for six leguminous crops across developmental stages and agronomic management systems. Faba bean produced the highest saponin concentrations and strongest biological activities, while mung bean occupied the opposite end of both spectra. The pod-filling stage represents the optimal harvest window for maximum saponin yield across all species. Organic management consistently enhanced saponin content relative to conventional cultivation, providing added functional value to organically produced legumes. The parallel increase in both beneficial and hemolytic activities with rising saponin content highlights the need for cultivar-specific approaches that optimize the ratio of desirable to undesirable saponin species. These quantitative datasets should aid food scientists and plant breeders in developing legume products and varieties with targeted bioactive profiles suited to specific nutritional and pharmaceutical applications.

Acknowledgements

Funding Sources: This research was funded by the Swiss National Science Foundation through the Agri-Food Research Program.

Institutional Support

The author acknowledges Bern Institute of Agricultural Sciences for access to the field station, greenhouse facilities, and analytical chemistry laboratory.

Contributions Not Qualifying for Authorship

Thanks are due to Ms. Julia Steiner for technical assistance with HPLC analysis and Mr. Thomas Brunner for field plot management.

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