

Evaluation of Isoflavone Extract from *Glycine max*: Spectral Characterization and Osteogenic Potential

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Abstract - Osteoporosis is a chronic, progressive skeletal disorder marked by low bone mineral density and micro-architectural deterioration, affecting an estimated 6.3% of men and 21.2% of women over 50 years worldwide and contributing to more than 8.9 million fractures annually. Conventional hormone replacement therapy carries notable long-term risks, driving interest in plant-derived phytoestrogens as safer alternatives for bone-health management. Soybean (*Glycine max* (L.) Merr.) is a rich dietary source of isoflavones (genistein, daidzein and glycitein), compounds structurally similar to endogenous estrogen and reported to influence osteoblast activity. The present study was undertaken to extract isoflavones from *Glycine max* seeds by continuous hot percolation (Soxhlet) using ethanol and by aqueous maceration, to screen the extracts phytochemically, and to evaluate cytotoxicity/cell-viability on the MG-63 (osteoblast-lineage) cell line by MTT assay as a preliminary indicator of osteogenic potential, supported by GC-MS profiling. The ethanolic extract gave a yield of 8.23% w/w and the aqueous extract 6-9.83% w/w. Preliminary screening confirmed the presence of alkaloids, phenolics, carbohydrates, saponins, terpenoids and amino acids, while steroids were absent. MTT assay on the MG-63 cell line showed a clear dose-dependent decline in cell viability with increasing extract concentration, yielding an IC₅₀ of 325.87 µg/mL. GC-MS analysis of the extract revealed multiple resolved peaks, including a cholesterol-related component, consistent with the presence of sterol and phytoestrogenic constituents. These findings support *Glycine max* isoflavones as a promising plant-based candidate for further osteogenic evaluation and eventual development as a natural alternative to conventional bone-health therapeutics.

Key Words: Osteoporosis; *Glycine max*; Isoflavones; Phytoestrogens; Genistein; Daidzein; Soxhlet extraction; Cytotoxicity; Osteogenic potential; Bone mineral density; Phytochemical screening; Natural alternative therapy

1. INTRODUCTION

Osteoporosis is a chronic, progressive skeletal disorder characterised by low bone mineral density and deterioration of bone micro-architecture, which together increase bone fragility and susceptibility to fracture. It is often termed a "silent disease" because bone loss occurs gradually and without pain until a fragility fracture—a break occurring from a fall at standing height or less—brings it to attention. Using WHO diagnostic criteria, osteoporosis is estimated to affect roughly 6.3% of men and 21.2% of women over the age of 50 globally, with Global Burden of Disease data reporting 41.5 million new cases in 2019, projected to rise to 263.2 million by 2030-2034. The disease causes more than 8.9 million fractures every year worldwide, with postmenopausal women disproportionately affected due to the accelerated bone loss that follows the decline in estrogen production.

Bone is a living tissue maintained by a continuous remodeling cycle involving osteoclasts (bone resorption), osteoblasts (bone formation) and osteocytes (mechanosensing), regulated by estrogen, parathyroid hormone, vitamin D, calcitonin and the RANKL/OPG signalling axis. After peak bone mass is attained in the late

twenties, resorption gradually outpaces formation; the sharp fall in estrogen at menopause markedly accelerates this imbalance, leading to trabecular thinning, cortical porosity and, ultimately, osteoporosis. Current pharmacological management includes antiresorptive agents (bisphosphonates, SERMs, denosumab, hormone therapy) and anabolic agents (parathyroid hormone analogues, sclerostin inhibitors), but long-term hormone-based therapy is associated with cardiovascular and oncological risks, prompting continued interest in safer, plant-derived alternatives.

Phytoestrogens-plant-derived compounds that structurally resemble 17β -estradiol-have attracted particular attention as candidates for bone-health support. Soybean (*Glycine max*) is among the richest dietary sources of isoflavones, principally genistein, daidzein and glycitein, which bind estrogen receptors and have been reported in several experimental and clinical studies to stimulate osteoblast differentiation, increase alkaline phosphatase activity and mineralisation, and suppress osteoclast-mediated resorption, while offering a more favourable long-term safety profile than conventional hormone replacement therapy. The present study was therefore designed to extract isoflavones from *Glycine max* seeds, characterise the extract phytochemically, and evaluate its cytotoxic/osteogenic behaviour on an osteoblast-lineage (MG-63) cell line, supported by GC-MS profiling of the extract.

2. PLANT PROFILE

Botanical name: *Glycine max* (L.) Merr. **Family:** Fabaceae **Part used:** Seed

Glycine max, commonly known as soybean, is an annual legume of the family Fabaceae, believed to have originated in China and domesticated from wild *Glycine soja*. It is now cultivated widely across Brazil, the USA, Argentina, China and India. The plant is a bushy, herbaceous annual, 30-100 cm tall, bearing trifoliate leaves and small self-pollinating flowers that develop into pods of 2-4 seeds, 5-11 mm in diameter. The seeds are notably rich in protein (36-40%) and oil (18-20%) and contain a diverse array of phytoconstituents, including the storage proteins glycinin and conglycinin, essential fatty acids, isoflavones (genistein, daidzein, glycitein), saponins (soyasaponins), phytosterols (β -sitosterol, stigmasterol, campesterol), oligosaccharides, dietary fibre, vitamins, minerals and bioactive peptides such as lunasin. These isoflavones act as phytoestrogens and underlie the seed's reported antioxidant, hypocholesterolemic, anticancer and bone-protective pharmacological significance, alongside traditional uses in supporting digestive, hepatic and menopausal health.



Fig. 1: Monograph of the Glycine max plant

3. LITERATURE REVIEW

A representative set of prior studies investigating the relationship between soy isoflavones and bone/osteoblast biology is summarised in Table 1. Collectively, this body of work indicates that soy-derived isoflavones and isoflavone-rich fractions consistently promote osteoblast differentiation and proliferation markers (ALP

activity, osteocalcin, mineralisation) in vitro and improve bone mineral density parameters in ovariectomised animal models and postmenopausal women, particularly at higher isoflavone doses, though the magnitude of benefit and optimal dosing remain areas of active investigation.

Author(s) / Year	Key Finding
Morris & Thorpe et al. (2006)	Genistein promoted a more organised osteoblast cytoskeleton in MG-63 cells and modulated cell-surface phosphatidylserine exposure, indicating osteogenic differentiation.
Wei & Liu et al. (2012)	Meta-analysis of RCTs found soy isoflavones increased bone mineral density by ~54% and reduced urinary deoxypyridinoline by 23% versus baseline, with greater benefit in postmenopausal women at >75 mg/day.
Yamaguchi & Gao-Balch et al. (2013)	Reviewed nutritional/food factors, including isoflavones, in regulating the osteoclast-osteoblast balance underlying age-related bone loss.
Fawwaz & Wahyudin et al. (2014)	Fermentation of soybean isoflavone glycosides to aglycones (genistein) enhanced osteoblast proliferation activity in vitro.
Choi & Choi et al. (2018)	Germinated soy germ extract increased ALP and osteocalcin expression via Runx2/Osterix activation through Smad signalling.
Shenoy, Bedi & Sandhu et al. (2023)	Soy protein isolate combined with resistance exercise improved bone-health parameters and muscle strength in osteopenic/osteoporotic postmenopausal women.
Li & Yang et al. (2023)	High-purity soy isoflavone aglycones improved bone parameters in an ovariectomised rat model of osteoporosis in a dose-dependent manner.
Gong & Zhang et al. (2025)	A hydrogel-delivered soybean-derived osteogenic peptide restored bone mineralisation in a glucocorticoid-induced zebrafish osteoporosis model, comparable to alendronate.
You & Wang et al. (2025)	Network meta-analysis comparing dairy and soy sources/components for bone-health effectiveness in women.
Aili & Chen et al. (2026)	Network pharmacology and MSC proliferation assays showed soybean isoflavone aglycones exerted greater osteogenic activity than their glycoside forms.

Table 1: Summary of relevant literature

4. AIM AND OBJECTIVES

Aim: To extract and evaluate the isoflavone content of Glycine max through spectral characterisation and to assess its osteogenic potential using an in vitro cell-line model.

Objectives:

- To extract isoflavones from Glycine max seeds by Soxhlet extraction using ethanol, and by aqueous maceration.
- To perform preliminary phytochemical screening confirming the presence of flavonoids/isoflavones and associated phytoconstituents.
- To characterise the extract using GC-MS for constituent profiling.
- To evaluate the cytotoxicity/cell-viability of the extract on the MG-63 osteoblast cell line using the MTT assay.
- To assess the osteogenic potential of the extract based on the dose-response viability profile as a preliminary indicator prior to further ALP/mineralisation studies.

5. MATERIALS AND METHODS

5.1 Collection and authentication of plant material

Fresh, healthy seeds of *Glycine max* (L.) Merr. were collected from agricultural fields in Dharmapuri, Tamil Nadu, during the month of August, at the appropriate maturity stage to obtain optimal phytochemical content. The material was identified and authenticated by Dr. S. Jagatheeskumar, Assistant Professor of Botany, based on comparison with standard reference material, and a voucher specimen was deposited at the herbarium of Sri Vijay Vidyalaya College of Arts and Science for future reference.

5.2 Preparation of plant material

Seeds were screened to exclude those with fungal contamination, insect infestation or physical damage, washed under running water, and shade-dried at room temperature to a constant weight to avoid thermal degradation of heat-sensitive phytoconstituents. The dried seeds were mechanically ground and passed through a 40-mesh sieve, and the resulting fine powder was stored in an airtight, light-protected container until extraction.

5.3 Extraction

Fifty grams of the powdered seed material was packed in a Soxhlet apparatus and subjected to continuous hot percolation with ethanol (60-70%) for 72 hours. Following completion, the solvent was removed under reduced pressure to yield a pale-yellow to light-golden residue, which was stored in a desiccator. In parallel, dried seed powder was extracted with distilled water/chloroform for 72 hours by cold maceration to obtain an aqueous extract (dark-green residue), which was similarly desiccated and packed in airtight containers for further study.



Fig. 2: Soxhlet apparatus used for extraction

5.4 Preliminary phytochemical screening

The ethanolic and aqueous extracts were subjected to standard qualitative tests for the principal phytoconstituent classes: alkaloids (Dragendorff's test), phenolics (ferric chloride test), carbohydrates (Molisch's test), saponins (foam test), steroids (Liebermann-Burchard test), terpenoids (Salkowski test) and amino acids (Ninhydrin test).



Fig. 3: Preliminary phytochemical test reactions

5.5 MTT cytotoxicity/cell-viability assay

The osteoblast-lineage MG-63 cell line (obtained from NCCS, Pune) was cultured in Dulbecco's Modified Eagle Medium (DMEM) with Ham's F-10, supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic solution, and maintained at 37 °C in a humidified incubator with 5% CO₂. Cells were seeded in 96-well plates and allowed to adhere for 24 hours, then treated with the extract at five concentrations (100, 200, 300, 400 and 500 µg/mL) alongside an untreated control, and incubated for a further 24 hours. MTT reagent (0.5 mg/mL final concentration) was added and the plate incubated in the dark for 3 hours; the resulting formazan crystals were solubilised in DMSO and absorbance measured at 570 nm using a microplate reader. Cell viability (%) was calculated as [mean absorbance of treated cells / mean absorbance of untreated cells] × 100, and the IC₅₀ value was derived from the dose-response curve.

5.6 GC-MS analysis

The ethanolic extract was subjected to gas chromatography-mass spectrometry to profile its constituent compounds, with peaks identified by retention time and matched to library spectra to support the phytochemical and osteogenic-relevance interpretation of the extract.

6. RESULTS AND DISCUSSION

6.1 Extractive yield

The continuous hot percolation (Soxhlet) method with ethanol gave a yield of 8.23% w/w, while the aqueous extract yielded 6% w/w by continuous percolation and 9.83% w/w by cold maceration (Table 2), reflecting the differing polarity-dependent solubility of the seed's phytoconstituents in each solvent system.

Plant / Part used	Method of extraction	Solvent	Yield (% w/w)
Glycine max, dried seed	Continuous hot percolation (Soxhlet)	Ethanol	8.23
Glycine max, dried seed	Continuous hot percolation (Soxhlet)	Aqueous	6.00
Glycine max, dried seed	Cold maceration	Aqueous	9.83

Table 2: Extractive yield of Glycine max seed extracts

6.2 Preliminary phytochemical screening

Both extracts tested positive for alkaloids, phenolics, carbohydrates, saponins, terpenoids and amino acids, while steroids were absent in either extract (Table 3). The consistent presence of phenolic and saponin constituents across both polarities is in line with the expected isoflavone (a phenolic/flavonoid subclass) and soyasaponin content of Glycine max seed.

Phytoconstituent	Test performed	Ethanolic extract	Aqueous extract
Alkaloids	Dragendorff's test	+	+

Phenolics	Ferric chloride test	+	+
Carbohydrates	Molisch's test	+	+
Saponins	Foam test	+	+
Steroids	Liebermann-Burchard test	-	-
Terpenoids	Salkowski test	+	+
Amino acids	Ninhydrin test	+	+

Table 3: Preliminary phytochemical screening of *Glycine max* seed extracts

6.3 MTT assay - cell viability of MG-63 cells

Treatment of MG-63 cells with the extract produced a clear, concentration-dependent decline in cell viability (Table 4, Fig. 4). Viability fell from 95.68% at 100 µg/mL to 14.64% at 500 µg/mL relative to the untreated control, giving an IC₅₀ of 325.87 µg/mL. This dose-response profile indicates that the extract is well tolerated by osteoblast-lineage cells at lower concentrations, a favourable feature when considering it as a candidate for promoting, rather than impairing, osteoblast viability, while higher concentrations exert a cytotoxic effect that should guide dose selection in subsequent osteogenic-marker (ALP, Alizarin Red S mineralisation) studies.

Concentration of extract (µg/mL)	Cell viability (%)
Untreated (control)	100.00
100	95.68
200	75.41
300	52.06
400	38.03
500	14.64

IC₅₀ value = 325.87 µg/mL

Table 4: MTT assay - % cell viability of MG-63 cells at increasing extract concentration

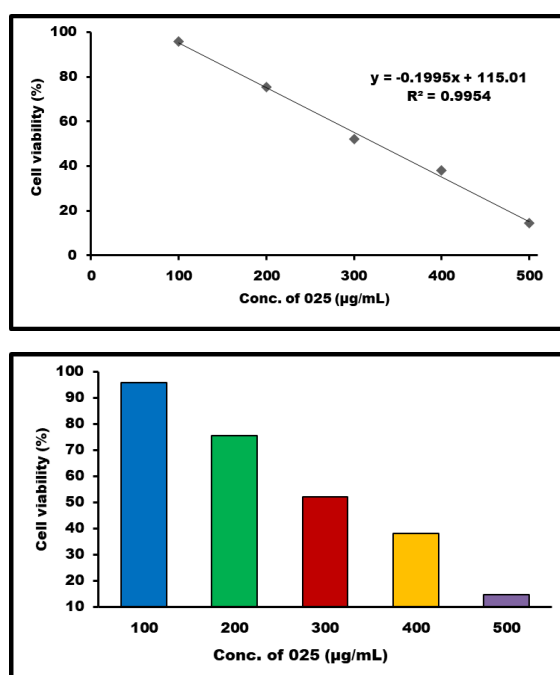


Fig. 4: Dose-response cell-viability curves of the *Glycine max* extract on MG-63 cells (MTT assay)

6.4 GC-MS profiling

GC-MS analysis of the ethanolic extract resolved multiple peaks across the chromatographic run (Fig. 5), with representative components summarised in Table 5. Notably, a peak corresponding to cholesterol (retention time ~19.68 min, area 3.68%) was identified alongside several nitrogen- and silicon-containing derivatives typical of derivatised phytosterol/steroid-related GC-MS profiles, consistent with the sterol and phytoestrogenic character expected of a soy seed extract.

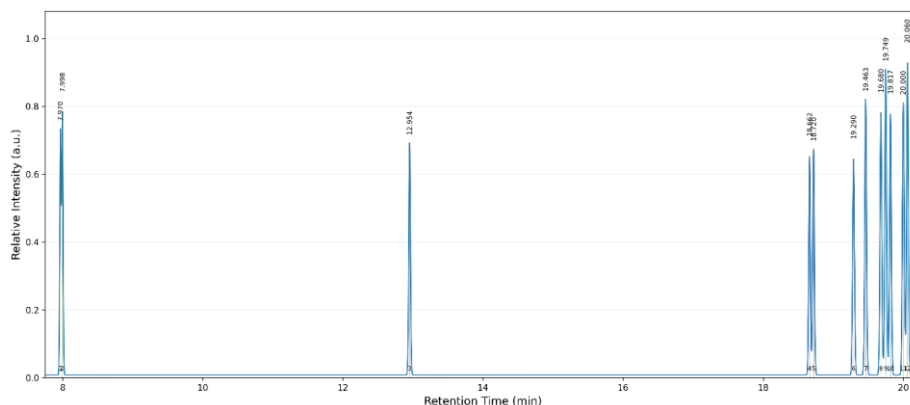


Fig. 5: GC-MS chromatogram of the ethanolic extract of Glycine max seed

Peak	R. Time (min)	Area %	Height %	Tentative compound
1	7.970	3.12	4.63	Phosphoric acid, bis[2,3-bis[(trimethylsilyl)oxy]propyl] derivative
2	7.998	2.52	4.95	Azastreptonigrin, isopropylidene-
6	19.290	10.68	4.12	Androstan-17-one, 3-(formyloxy)-11-hydroxy- derivative
8	19.680	3.68	5.00	Cholesterol
11	20.000	5.51	5.19	Chlorinated cyclohexenyl-ethoxyphenyl derivative

Table 5: Representative peaks from GC-MS analysis of the ethanolic extract

6.5 Discussion

The extractive yield and consistent phytochemical profile confirm that both polar (aqueous) and moderately non-polar (ethanolic) solvent systems recover the principal bioactive constituents of Glycine max seed, with phenolics and saponins-classes that encompass the isoflavones and soyasaponins reported in the literature to confer phytoestrogenic and cholesterol-modulating activity-detected in both extracts. The MTT assay result is consistent with prior reports (Morris & Thorpe, 2006; Fawwaz & Wahyudin, 2014; Choi & Choi, 2018) that soy-derived isoflavone fractions are well tolerated by osteoblast-lineage cells at moderate concentrations, supporting a concentration window within which the extract may be evaluated for pro-osteogenic markers such as ALP activity and Alizarin Red S mineralisation in future work. The GC-MS detection of a cholesterol-related component further supports the presence of sterol-class constituents structurally related to the phytoestrogenic isoflavones associated with improved bone mineral density in the reviewed literature.

7. CONCLUSION

The present study successfully extracted and characterised isoflavone-rich fractions from Glycine max (soybean) seed by Soxhlet (ethanolic) and maceration (aqueous) extraction. Preliminary phytochemical screening confirmed the presence of alkaloids, phenolics, carbohydrates, saponins, terpenoids and amino acids, while GC-MS profiling detected a cholesterol-related constituent consistent with the expected sterol/phytoestrogen content of the seed. The MTT assay on the MG-63 osteoblast-lineage cell line

demonstrated a clear, concentration-dependent viability profile with an IC₅₀ of 325.87 µg/mL, indicating that the extract is well tolerated at lower concentrations-supporting its suitability for further osteogenic evaluation. Taken together, these findings support Glycine max-derived isoflavones as a promising, plant-based candidate for bone-health management, offering a potentially safer profile than conventional hormone replacement therapy, particularly for postmenopausal women. In vivo studies, dedicated osteogenic-marker assays (ALP activity, mineralisation), bioavailability assessment and eventual clinical evaluation are warranted to substantiate the therapeutic potential indicated by this preliminary work.

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