



Original Article

Preliminary Phytochemical Screening of *Glycine max* (L.) Merr. Residues: A Qualitative Assessment for Potential Valorization

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Abstract:

Agricultural residues produced during crop cultivation and harvesting constitute an underexploited biomass resource that may contain chemically and biologically important constituents. The present study was designed to establish a preliminary qualitative phytochemical profile of *Glycine max* (L.) Merr. residues and to examine their potential for further valorization-based research. The residue material was cleaned, shade-dried, powdered, and separately extracted with ethanol, methanol, and distilled water. The prepared extracts were evaluated through conventional qualitative phytochemical tests. The recorded observations showed detectable reactions for alkaloids, carbohydrates, reducing sugars, proteins, flavonoids, phenolic compounds, tannins, saponins, coumarins, and gums and mucilage in one or more extracts. Cardiac glycosides, phlobatannins, phytosterols, terpenoids, quinones, and resins were not detected under the conditions employed. Strong alkaloid- and protein-related reactions were observed in all three extracts, whereas reducing-sugar reactions were relatively stronger in methanolic and aqueous extracts. Flavonoid reactions were comparatively more pronounced in the ethanolic extract, while tannin responses were stronger in methanolic and aqueous extracts. Overall, the findings provide an initial chemical basis for further investigation of soybean residues. Nevertheless, the qualitative assays cannot confirm the identity, concentration, purity, or biological activity of individual constituents. Quantitative measurements and chromatographic or spectrometric characterization, followed by suitably controlled bioactivity studies, are necessary before functional, nutraceutical, or pharmaceutical applications can be considered.

Keywords: *Glycine max*; Soybean Residues; Preliminary Phytochemical Screening; Phytoconstituents; Qualitative Analysis; Agricultural Residues; Valorization.

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Introduction:

Crop production, harvesting, threshing, and processing generate considerable amounts of plant-derived biomass. Materials such as leaves, stems, pod husks, seed coats, and other crop residues are often discarded, burned, or utilized for low-value purposes. Their conversion into higher-value products is receiving increasing attention as part of sustainable biomass management and circular-bioeconomy strategies. Since such residues may contain carbohydrates, proteins, phenolic compounds, and other secondary metabolites, systematic characterization is justified before they are regarded simply as waste.

Glycine max (L.) Merr., commonly referred to as soybean, is an economically significant legume and oilseed crop. Although its seeds are mainly valued as sources of protein and oil, soybean cultivation and harvesting also produce considerable quantities of above-ground biomass. Soybean-derived materials contain various phytochemical groups, including phenolic compounds, flavonoids, isoflavonoids, and saponins. The distribution and extractability of these constituents may vary with the plant part, cultivar, stage of maturity, environmental conditions, processing history, and solvent system. Consequently, screening defined residue fractions can generate useful preliminary information for subsequent targeted chemical investigations.

Preliminary qualitative phytochemical screening is an inexpensive exploratory method for detecting broad groups of compounds in crude plant extracts. These tests can assist in sample selection and in planning subsequent analytical work, but their results are presumptive rather than conclusive. A colour or precipitation reaction generally cannot establish

the presence of a particular molecule, while a negative reaction cannot demonstrate that an entire compound class is completely absent. More definitive characterization can subsequently be undertaken using instrumental approaches such as TLC/HPTLC, GC-MS, LC-MS, FTIR, and NMR.

Accordingly, the present investigation compared the preliminary phytochemical responses of ethanol, methanol, and aqueous extracts prepared from *Glycine max* residues using conventional qualitative assays. The study is intended as an initial screening exercise that can provide a foundation for subsequent quantitative and instrumental characterization.

Aim of the Study:

To evaluate the preliminary qualitative phytochemical composition of *Glycine max* (L.) Merr. residues extracted using ethanol, methanol, and distilled water, and to identify phytochemical groups that warrant further characterization.

Objectives:

- To collect and prepare soybean residue material for laboratory investigation.
- To obtain ethanol, methanol, and aqueous extracts from the residue material.
- To qualitatively screen the prepared extracts for selected phytoconstituent classes.
- To compare the qualitative response patterns obtained from the three solvent extracts.
- To determine phytochemical groups that require further quantitative and chromatographic or spectrometric examination.



- To preliminarily evaluate the research potential of soybean residues for value-added utilization.

Materials and Methods:

1. Study Area and Collection:

The investigation was linked with soybean-growing agricultural areas of the Amravati region of Maharashtra, India. The supplied study record refers to the Daryapur subdivision of Amravati district, including Mahuli Dhande village, in the earlier crop-residue investigation. The collected soybean residue material was subsequently transported to the laboratory for processing and analysis.

2. Plant Material and Identification:

Residue material of *Glycine max* (L.) Merr. obtained during soybean cultivation and harvesting was used for the study. The selected material was transported to the laboratory for further processing. plant part or parts analyzed—such as leaves, stems, pod husks, seed coats, or a defined mixture—should also be specified from the original laboratory record.

3. Cleaning, Drying and Powdering:

The collected residue was manually cleaned to eliminate adhering soil, dust, and other foreign materials. After cleaning, the material was shade-dried under ambient conditions with sufficient ventilation until a constant weight was obtained. The dried residue was mechanically pulverized to obtain a comparatively uniform powder and transferred to clean, dry, airtight amber-coloured containers for storage under cool conditions until extraction. Shade drying was adopted to reduce the possibility of degradation of constituents that may be sensitive to heat or light.

4. Preparation of Extracts:

4.1 Successive Solvent Extraction:

- a. Weighed 400 g of powdered plant residues into a clean, dry round-bottom flask.
- b. Performed successive extraction in Soxhlet apparatus with solvents of increasing polarity: Ethanol, Methanol (polar) and distilled water. Extracted each solvent for 6–8 hours or until siphoning becomes colorless.
- c. Concentrated each solvent extract under reduced pressure using a rotary evaporator at solvent-appropriate temperature (Ethanol 40–60 °C, Methanol 45–50 °C) to dryness. For aqueous fraction, evaporate at ≤ 40 °C.
- d. Recorded the weight of each dried extract and calculate percent yield: Percent yield (%) = (weight of dried extract (g) / weight of starting powdered material (g)) $\times$ 100. Store extracts at 4 °C in amber vials until phytochemical testing.

5. Preliminary Qualitative Phytochemical Screening:

The ethanol, methanol, and aqueous extracts were examined using conventional qualitative phytochemical assays. Approximately 10 mg of each extract was reported to have been dissolved in a suitable solvent for the respective tests, with appropriate controls used where applicable. The screening included tests for alkaloids, carbohydrates, reducing sugars, cardiac glycosides, proteins, flavonoids, phenolic compounds, tannins, phlobatannins, saponins, phytosterols, terpenoids, quinones, coumarins, gums and mucilage, and resins.

The names of the tests and the recorded observations were retained from the supplied



experimental record. Since these conventional assays are presumptive in nature, a positive result is interpreted as a detectable reaction associated with a broad chemical class rather than as definitive proof of a particular compound. Relevant phytochemical references and quality-control recommendations should be cited together with the exact procedures followed in the laboratory.

6. Interpretation of Qualitative Responses:

In the supplied observation table, the symbols +, ++, and +++ denote lower, moderate, and stronger visual reactions, respectively, whereas – indicates a negative reaction. These symbols are considered qualitative or semi-qualitative observations and

should not be treated as measurements of concentration or relative quantitative abundance. The criteria used to assign the reaction-intensity scores should be documented from the original laboratory records before publication. If no predefined scoring criteria were used, reporting the findings simply as 'detected' or 'not detected' would be the more appropriate journal presentation.

Results:

Qualitative phytochemical screening of the Glycine max residue extracts revealed variation in the observed reaction intensities among the ethanol, methanol, and distilled-water extracts.

The observations provided for the present manuscript are compiled in Table 1.

No.	Phytochemical Group	Test Reported	Ethanol	Methanol	Distilled Water
1	Alkaloids	Wagner's test	+++	+++	+++
2	Carbohydrates	Starch test	+	+	+
3	Reducing sugars	Fehling's test	++	+++	+++
4	Cardiac glycosides	Keller–Kiliani test	–	–	–
5	Proteins	Xanthoproteic test	+++	+++	+++
6	Flavonoids	Lead acetate test	++	+	+
7	Phenolic compounds	Lead acetate test	+	+	+
8	Tannins	10% NaOH test	+	++	++
9	Phlobatannins	HCl test	–	–	–
10	Saponins	Froth test	+	+	+
11	Phytosterols	Salkowski/Hesse's response	–	–	–
12	Terpenoids	Salkowski test	–	–	–
13	Quinones	Alcoholic KOH test	–	–	–
14	Coumarins	NaOH test	++	++	+
15	Gums and mucilage	Alcohol test	+	+	+
16	Resins	Acetic anhydride test	–	–	–

Note: + = lower qualitative response; ++ = moderate response; +++ = stronger response; – = not detected under the stated test conditions. The scoring system is qualitative and does not represent quantitative concentration.



1. Alkaloids:

Wagner's test gave a positive reaction in all three extracts, and the supplied observation sheet recorded +++ for ethanol, methanol, and aqueous extracts. This finding should be understood as a strong response within the qualitative assay used and should not be interpreted as evidence of a high alkaloid concentration or as identification of particular alkaloid compounds.

2. Carbohydrates and Reducing Sugars:

Positive carbohydrate-related reactions were obtained in all three extracts using the reported starch test. Reducing sugars were also detected in each extract, with an intensity of ++ in ethanol and +++ in both methanol and distilled-water extracts. Because the starch test does not quantify total carbohydrate content, the finding is presented only as a positive reaction under the specified assay conditions.

3. Cardiac Glycosides:

The Keller–Kiliani test did not produce a positive reaction for cardiac glycosides in any of the three extracts.

4. Proteins:

The Xanthoproteic test yielded +++ reactions for ethanol, methanol, and aqueous extracts. This observation indicates the presence of protein-related constituents detectable by the applied reaction; it should not, however, be interpreted as a quantitative determination of total protein.

5. Flavonoids and Phenolic Compounds:

Flavonoid reactions were positive in all three extracts, with a comparatively stronger response in ethanol (++), whereas methanol and aqueous extracts showed + reactions. Phenolic compounds also produced positive (+) responses in all three extracts. These results demonstrate detectable reactions associated

with these broad classes but do not establish the identity of individual flavonoid or phenolic compounds.

6. Tannins, Saponins and Coumarins:

Tannins were detected in each extract, with a + response in ethanol and ++ responses in methanol and distilled water. Saponins gave positive (+) results in all three extracts in the froth test. Coumarins were likewise detected in all extracts, producing ++ responses in ethanol and methanol and a + response in the aqueous extract.

7. Other Constituents:

The supplied observations did not show detectable reactions for phlobatannins, phytosterols, terpenoids, quinones, or resins. Emodins were not included in the results table because the original record stated 'not recorded'; therefore, no interpretation or conclusion is made concerning emodins.

Comparative Phytochemical Profile:

The recorded observations demonstrate several common qualitative responses among the three solvent extracts. Alkaloid- and protein-related reactions were assigned +++ in ethanol, methanol, and aqueous extracts. Reducing-sugar responses were relatively stronger in methanol and distilled water. The flavonoid reaction was comparatively stronger in ethanol, whereas tannin responses were more pronounced in methanol and water. Coumarin responses were comparatively stronger in ethanol and methanol. Positive reactions associated with carbohydrates, phenolic compounds, saponins, and gums and mucilage were observed across all three extracts.

These observations represent differences in assay-response patterns and should not be interpreted as a quantitative



ranking of phytochemical levels. Solvent polarity, extraction conditions, and the chemical composition of the plant matrix can influence both the recovery of constituents and the intensity of qualitative reactions.

Discussion:

The present investigation establishes a preliminary qualitative chemical profile of extracts prepared from soybean residues. The detection of phenolic compounds, flavonoids, tannins, and saponins agrees broadly with the phytochemical composition reported for soybean-derived materials. Soybean is known to contain several groups of bioactive constituents, including phenolic acids, flavonoids or isoflavonoids, and saponins; however, their distribution can differ considerably between tissues and cultivars. Thus, the current observations justify further investigation but cannot be used to estimate the concentrations of these constituents in the analyzed residue.

The relatively stronger reducing-sugar response observed in methanol and water is chemically reasonable because many low-molecular-weight sugars and related polar constituents are more readily associated with polar extraction media. However, qualitative colour or precipitation reactions may be affected by matrix effects and reagent specificity. Accordingly, solvent-related differences should be regarded as observations that require confirmation through quantitative analytical procedures.

The positive Wagner's-test response in all three extracts should likewise be interpreted with caution. Classical colour and precipitation assays are valuable preliminary screening tools, but nonspecific reactions may occur in complex crude extracts. Confirmation of alkaloids,

flavonoids, phenolic compounds, saponins, or coumarins requires chromatographic and/or spectrometric analysis using appropriate standards.

Negative observations also require careful interpretation. The absence of a reaction under a particular test condition does not demonstrate the absolute absence of the corresponding chemical class. Detection may depend on extraction efficiency, analyte concentration, molecular structure, matrix effects, reagent specificity, and assay sensitivity. Accordingly, the negative findings in this study are most appropriately described as 'not detected under the conditions used.'

The principal scientific contribution of the study is therefore exploratory, as it identifies residue extracts and broad chemical classes that can be prioritized for subsequent analytical work. Additional investigations involving total phenolic and total flavonoid assays, targeted estimation of saponins or other relevant constituents, TLC/HPTLC fingerprinting, GC-MS or LC-MS profiling, and suitably designed antioxidant and antimicrobial assays would provide stronger evidence for valorization. Such future studies should incorporate appropriate analytical controls, biological or technical replicates, and relevant statistical analysis.

Potential Valorization Perspective:

The preliminary detection of multiple phytochemical classes indicates that soybean residues may warrant further investigation as a source of recoverable compounds. A systematic valorization strategy could include residue identification and collection, standardized drying and milling, optimization of extraction, qualitative screening, chromatographic fingerprinting, targeted quantitative



determination, compound identification, safety assessment, and biological validation.

Possible research avenues include the recovery of phenolic- or flavonoid-rich fractions, characterization of saponin-containing fractions, preparation of standardized residue extracts, and evaluation of antioxidant or antimicrobial properties. These should be regarded as prospective research directions rather than established applications. Any nutraceutical, pharmaceutical, cosmetic, or antimicrobial claim would require compound-level characterization, validated bioassays, toxicological and safety evaluation, and appropriate regulatory assessment.

Soybean cultivation → Residue generation → Collection → Cleaning → Drying → Powdering → Standardized extraction → Qualitative screening → TLC/HPTLC fingerprinting → GC-MS/LC-MS/other characterization → Quantitative analysis → Bioactivity and safety evaluation → Evidence-based valorization

Conclusion:

The present preliminary study demonstrates that *Glycine max* (L.) Merr. residue extracts prepared with ethanol, methanol, and distilled water produce a range of qualitative phytochemical reactions. Strong alkaloid- and protein-related responses were recorded in all three extracts, whereas reducing-sugar reactions were comparatively more pronounced in methanol and distilled water. Flavonoid response was relatively stronger in ethanol, while tannin response was comparatively stronger in methanol and water. Phenolic compounds, saponins, coumarins, and gums and mucilage were also detected in the examined extracts. Cardiac glycosides,

phlobatannins, phytosterols, terpenoids, quinones, and resins were not detected under the reported experimental conditions.

Taken together, these findings provide preliminary support for further chemical investigation of soybean residues as an underutilized biomass resource. The observations do not, however, establish the concentration, molecular identity, purity, safety, or biological effectiveness of the detected constituents. Future investigations should therefore incorporate standardized extraction procedures, quantitative phytochemical assays, chromatographic and spectrometric characterization, and appropriately controlled biological and safety assessments before proposing practical value-added applications.

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