

Evaluation of the Antioxidant and Antibacterial Activities of Solvent Extracts of *Glycine max* (L.) Merr. Residues

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Abstract

Soybean (*Glycine max* (L.) Merr.) residues may serve as a potential source of naturally occurring compounds with antioxidant and antibacterial properties. The present study investigated the antioxidant activity and preliminary antibacterial potential of methanolic, ethanolic, n-hexane and distilled-water extracts of soybean residues. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay at seven concentrations ranging from 0.7 to 50 µg/mL, with ascorbic acid used as the reference antioxidant. According to the supplied graph, the n-hexane extract showed the highest estimated radical-scavenging activity at 50 µg/mL (approximately 82–83%), followed by the ethanolic extract (68–69%), methanolic extract (approximately 53%) and aqueous extract (approximately 46%). These values should be considered approximate because the original absorbance readings were unavailable for independent verification. Preliminary antibacterial screening was conducted against *Staphylococcus aureus* NCIM 2925 and *Escherichia coli* NCIM 2065 using the agar-well diffusion method, with streptomycin as the positive control. A resazurin-based microdilution assay was also reported. However, incomplete methodological details and the absence of results separated by bacterial species prevented reliable quantitative comparisons of inhibition zones and confirmation of organism-specific minimum inhibitory concentrations (MICs). Overall, the available findings indicate differences in the observed DPPH response among the solvent extracts. Further investigation using standardized experimental procedures and complete data records is necessary to validate these observations and assess the potential value of soybean residues for sustainable utilization.

Keywords: soybean residues; *Glycine max*; DPPH assay; antioxidant activity; antibacterial activity; resazurin assay; agricultural residue utilization

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is an economically valuable leguminous crop widely used in food production, animal feed and various industrial applications. In addition to its nutritional importance, soybean-derived materials have received attention for their phytochemical composition and potential antioxidant properties. Malenčić et al. (2007) investigated the phenolic content and antioxidant properties of soybean seeds, providing useful background for research on soybean phytochemicals. However, findings obtained from seeds cannot necessarily be applied to agricultural residues because the chemical composition may vary among different plant parts and processing by-products. In a related study,

Cabezudo et al. (2021) examined the recovery of polyphenols from soybean hulls, demonstrating the research interest in utilizing soybean-processing residues as potential sources of valuable compounds. Agricultural residues produced during cultivation and processing are often underutilized. Their valorization involves exploring ways to recover useful constituents or identify potential applications for materials that might otherwise be discarded. Investigating the chemical composition and biological properties of these residues is an important initial step in determining their possible value. In-vitro antioxidant assays are frequently used to assess the ability of extracts to react with free radicals under defined experimental conditions. Nevertheless, antioxidant activity observed in a laboratory assay does not automatically indicate the same effect in living organisms.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a widely used method for evaluating the radical-scavenging capacity of plant extracts. It is based on the reduction of the coloured DPPH radical by compounds capable of donating electrons or hydrogen atoms, which produces a measurable change in absorbance (Brand-Williams et al., 1995). The results may vary depending on factors such as solvent composition, sample concentration, reaction time, wavelength and the procedure used for blank correction and calculation. Different antioxidant assays also operate through different chemical mechanisms and may produce different types of results. Therefore, the findings should be interpreted according to the specific method used (Prior et al., 2005).

The solvent selected for extraction can substantially influence the composition of a crude plant extract. Methanol, ethanol, n-hexane and water differ in their polarity and ability to dissolve particular constituents from plant material. Consequently, extracts prepared using these solvents may exhibit different chemical profiles and biological responses. Such variation may be associated with extraction efficiency, compound solubility, extract composition and interactions within the assay system. However, a higher response in a particular extract does not, by itself, establish the identity or chemical nature of the compounds responsible. Additional phytochemical characterization is required to investigate the constituents associated with the observed activity.

Antibacterial screening is another useful approach for the preliminary evaluation of plant-derived extracts. It helps determine whether an extract can inhibit bacterial growth under specified laboratory conditions. The agar-well diffusion method is commonly employed for initial screening, while broth microdilution can be used to determine the minimum inhibitory concentration (MIC) when the experimental conditions and endpoint criteria are properly established. The interpretation of agar-well diffusion results requires caution because inhibition-zone size can be influenced by the ability of extract constituents to diffuse through the agar, in addition to their antibacterial properties (Balouiri et al., 2016). Although plant extracts may contain several classes of compounds with potential antimicrobial activity, laboratory observations alone are insufficient to establish their practical or therapeutic effectiveness (Cowan, 1999).

Considering the potential value of agricultural by-products, the present study examines the reported antioxidant and preliminary antibacterial properties of four solvent extracts of soybean residues. The antioxidant response was evaluated using the DPPH radical-scavenging assay, while antibacterial screening was reported against *S. aureus* NCIM 2925 and *E. coli* NCIM 2065. The available resazurin microdilution results were also considered, taking into account the limitations of the supporting experimental records. As the supplied documentation does not consistently identify the specific soybean residue fraction used, the term “soybean residues” is retained until the original sample records confirm whether the material consisted of leaves, stems, pod husks, seed coats or another plant part.

2. Aim and objectives

The aim was to evaluate the in-vitro antioxidant and antibacterial potential of different solvent extracts of soybean residues.

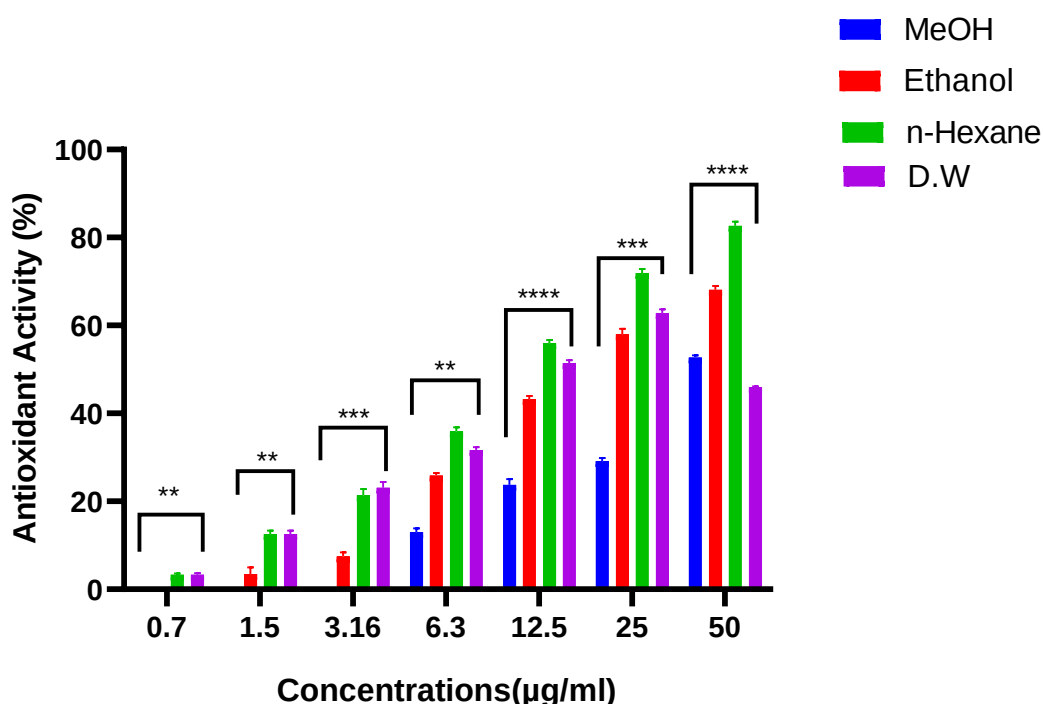
- To assess DPPH radical-scavenging responses of methanolic, ethanolic, n-hexane and distilled-water extracts across the reported concentration range.
- To screen the four extracts for antibacterial activity against *S. aureus* NCIM 2925 and *E. coli* NCIM 2065 using agar-well diffusion.
- To document the reported resazurin microdilution results while identifying missing information that prevents reliable organism-specific MIC assignment.
- To consider the relevance of the findings to further research on soybean-residue valorization without overstating preliminary in-vitro evidence.

3. Antioxidant activity (DPPH assay)

DPPH free radical scavenging activity of samples were individually dissolved in water to prepare stock solutions at 6.0 mg/mL. A series of seven concentrations was generated through serial dilution. Each solution (60 μ L) was added to a 96-well plate, followed by 200 μ L of 0.004% DPPH in ethanol. The mixture was incubated at 37 °C for 5 min, and absorbance was measured at 520 nm. Controls included a negative control (water) and a blank (ethanol instead of DPPH solution). Ascorbic acid was used as a standard reference (positive control). All experiments were performed in triplicate, and DPPH radical scavenging activity was calculated using the following formula: DPPH free radical scavenging activity (Brand-Williams et al., 1995; Prior et al., 2005).

where A control is the negative control, A blank is the blank control and A sample is the measured value of the sample.

Result



3.1 Result

The antioxidant potential of different solvent extracts was evaluated using the DPPH radical scavenging assay. All extracts exhibited a concentration-dependent increase in antioxidant activity over the tested concentration range (0.7–50 µg/mL), indicating progressive free radical scavenging with increasing extract concentration.

Among the tested extracts, the n-hexane extract consistently showed the highest antioxidant activity at almost all concentrations, reaching approximately 82–83% radical scavenging at 50 µg/mL. The ethanolic extract demonstrated the second-highest activity, attaining about 68–69% inhibition at the highest concentration. The methanolic extract exhibited moderate antioxidant activity, reaching nearly 53% scavenging at 50 µg/mL, whereas the distilled water (D.W.) extract showed comparatively lower activity, with a maximum inhibition of approximately 46% at the same concentration.

At lower concentrations (0.7–3.16 µg/mL), all extracts displayed relatively weak antioxidant activity, with only slight differences among solvents. However, from 6.3 µg/mL onward, a marked increase in DPPH radical scavenging was observed, particularly for the n-hexane and ethanolic extracts, indicating that these solvent systems were more efficient in extracting antioxidant constituents. The methanolic extract showed a gradual increase in activity, while the aqueous extract exhibited moderate improvement but remained less effective than the organic solvent extracts throughout the tested concentration range.

The superior antioxidant activity of the n-hexane extract suggests that a substantial proportion of the antioxidant compounds present in the plant may be relatively non-polar in nature. The higher activities observed in the organic solvent extracts compared with the aqueous extract further emphasize the influence of solvent polarity on the extraction efficiency of bioactive antioxidant metabolites.

Statistical analysis revealed significant differences in antioxidant activity among the extracts at increasing concentrations, as indicated by the significance levels shown in the figure ($p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$). These findings demonstrate that the n-hexane extract possessed significantly greater DPPH radical scavenging activity than the other solvent extracts, particularly at higher concentrations.

4. ANTIBACTERIAL ACTIVITY

Materials and method

Chemicals and media

Nutrient broth, nutrient agar (NA), Methanol, streptomycin sulphate (Hi Media, Mumbai, India), Resazurin (Sigma Aldrich, India). All compounds were dissolved in methanol and different concentrations were prepared. A bacterial species *E.coli* and *S.aureus* was procured from the National Collection of Industrial Microorganisms (NCIM) CSIR-NCL, India.

Bacterial strains and media

Bacterial strains *Escherichia coli* and *Staphylococcus aureus* were used in this study. Both the strains are cultured in Nutrient broth media at 110 rpm and 37 °C for 24 hours.

Antimicrobial activity

The antibacterial activity of the methanol (MeOH), n-hexane (n-hex), distilled water (DW), and ethanol (ET) extracts was evaluated using the agar well diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The extracts were screened against the Gram-positive bacterium *Staphylococcus aureus* NCIM 2925 and the Gram-negative bacterium *Escherichia coli* NCIM 2065. Briefly, bacterial strains were cultivated overnight in Mueller–Hinton broth and adjusted to match a 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU mL⁻¹). Mueller–Hinton

agar plates were uniformly seeded with the respective bacterial suspensions using sterile cotton swabs to form a bacterial lawn. Wells of 6 mm diameter were aseptically bored into the agar, into which 100 μL of each test extract (4 mg mL^{-1}) was loaded into designated wells (MeOH, n-hex, DW, and ET). Streptomycin (0.5 mg mL^{-1}) was added to the central well to serve as the positive control (+VE), while the pure extraction solvents were used as negative controls. After incubation at 37 $^{\circ}\text{C}$ for 18–24 h, antibacterial efficacy was assessed by measuring the zone of inhibition (mm) surrounding each well. All assays were executed in triplicate, and data were presented as mean $\pm$ standard deviation (SD). (Clinical and Laboratory Standards Institute [CLSI], 2024).

Well Diffusion Method.



Fig.6: Antimicrobial Activity by Well Diffusion Method

4.1 Resazurin assay (MIC determination)

Minimum Inhibitory Concentration (MIC) Assay

The minimum inhibitory concentration (MIC) of the methanol (MeOH), n-hexane (n-hex), distilled water (DW), and ethanol (ET) extracts was determined using the microdilution resazurin assay in 96-well flat-bottom transparent microtiter plates. Briefly, bacterial cultures of *Staphylococcus aureus* NCIM 2925 and *Escherichia coli* NCIM 2065 were grown overnight in Nutrient broth, and their turbidity was adjusted to an OD_{600} of 0.1. Each well was initially filled with 100 μL of Nutrient broth. The stock solutions of the extracts (4 mg mL^{-1} in methanol) were added to the first row of wells to achieve an initial concentration of 500 $\mu\text{g mL}^{-1}$, followed by serial two-fold dilutions across the plate to yield 100 μL per well. The medium containing the highest concentration of methanol was included as a solvent control to evaluate background solvent effect. Subsequently, 10 μL of the prepared bacterial suspension was inoculated into each well. The plates were incubated at 37 $^{\circ}\text{C}$ with shaking at 90 rpm for 18 h. Following incubation, 5 μL of resazurin solution (2 mg mL^{-1} in distilled water) was added to each well under dark conditions, and the plates were further incubated in the dark for 6 h. Bacterial growth was determined visually based on the color change of resazurin (blue indicating inhibition and pink indicating viable cell growth).

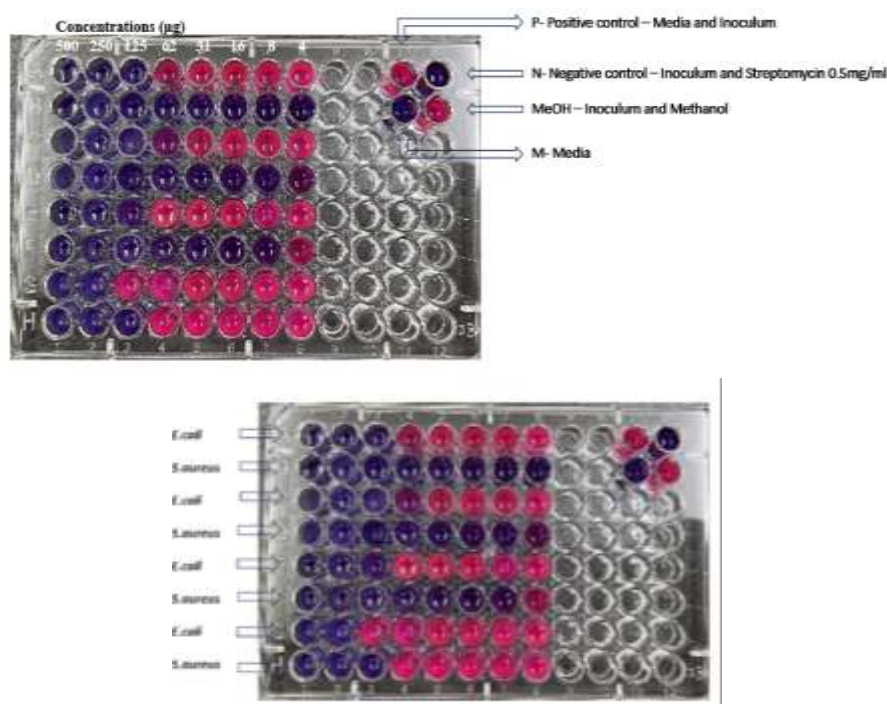


Fig: Minimum inhibitory concentration assay by Resazurin

Percent growth inhibition in E.coli and S.aureus

Compound s	Concentration (µg/ml)							
	500	250	125	62	31	16	8	4
n-Hexane	86.727 2	72.7722 8	56.6514 8	23.7621 8	19.2885 3	5.58136	4.5303	2.6199 5
MeOH	89.705 3	83.59711	81.7454 4	75.6349 5	69.6852 9	66.0741 2	66.1926 7	62.148 1
Ethanol	82.667 5	78.4114	73.2724 5	54.675	24.77116	15.5545 6	8.84219	4.9139
Distilled water	87.017 7	83.546	79.094	76.829	71.243	66.389	58.379	51.983

5. Results and Discussion

5.1. Antioxidant Activity of Glycine max Residue Extracts

The antioxidant potential of methanolic, ethanolic, n-hexane and distilled-water extracts of Glycine max residues was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay at concentrations ranging from 0.7 to 50 µg/mL. The supplied results indicated differences in radical-scavenging responses among the solvent extracts. According to the accompanying graph and narrative, the n-hexane extract exhibited the highest reported activity at 50 µg/mL, with approximately 82–83% radical scavenging, followed by the ethanolic extract (approximately 68–69%), methanolic extract (approximately 53%) and distilled-water extract (approximately 46%). These values are approximate estimates from the supplied report and have not been independently recalculated from raw absorbance data.

The reported results generally suggest increased radical-scavenging activity at higher extract concentrations, particularly for the n-hexane and ethanolic extracts. However, the aqueous extract graph appears to show a decrease at some higher concentrations, which does not fully agree with the narrative description of a consistent concentration-dependent increase. The original absorbance readings should therefore be checked before a definitive concentration-response relationship is claimed.

The comparatively high DPPH response reported for the n-hexane extract indicates that this extract displayed greater apparent radical-scavenging activity under the experimental conditions used. Differences among extracts may reflect solvent-dependent extraction efficiency, extract composition, solubility and possible matrix effects. Nevertheless, DPPH activity alone does not establish the chemical identity or polarity of the compounds responsible for the observed response. Further chemical characterization would be necessary to identify the constituents contributing to the activity. The DPPH assay is widely used for preliminary evaluation of radical-scavenging capacity, although its results depend on experimental conditions and calculation procedures (Brand-Williams et al., 1995).

Although significance symbols were included in the original antioxidant graph, the raw absorbance readings, replicate-level percentages, statistical test, post-hoc procedure and exact comparison groups were not available for independent verification. Consequently, the significance annotations cannot be considered independently confirmed statistical evidence. The antioxidant findings are therefore interpreted descriptively, and the reported differences should be treated as preliminary until the original data and statistical calculations have been verified.

5.2. Antibacterial Activity of Glycine max Residue Extracts

The antibacterial potential of the four solvent extracts—methanol, ethanol, n-hexane and distilled water—was investigated against the Gram-positive bacterium *Staphylococcus aureus* NCIM 2925 and the Gram-negative bacterium *Escherichia coli* NCIM 2065. The supplied report describes agar-well diffusion screening using extracts at 4 mg/mL, with streptomycin at 0.5 mg/mL as the positive control and the corresponding extraction solvents as negative controls. The assay was reported to have been performed in triplicate.

The supplied report includes a photograph of the agar-well diffusion assay, but numerical inhibition-zone diameters for individual extracts, bacterial strains and replicates were not provided. Therefore, the magnitude of antibacterial activity and the relative effectiveness of the four solvent extracts cannot be compared quantitatively from the available evidence. Agar-well diffusion results should also be interpreted in relation to extract solubility and diffusion through the agar medium, because visible inhibition zones do not depend solely on antibacterial potency (Balouiri et al., 2016).

A resazurin microdilution assay was also described to assess bacterial growth inhibition. Resazurin-based microtitre methods have been used to indicate bacterial growth during in-vitro screening of phytochemicals (Sarker et al., 2007). The accompanying table reports percentage-growth-inhibition values for the four extracts at concentrations ranging from 4 to 500 µg/mL. At 500 µg/mL, the reported inhibition values were 86.73% for the n-hexane extract, 89.71% for the methanolic extract, 82.67% for the ethanolic extract and 87.02% for the distilled-water extract. At 4 µg/mL, the corresponding reported values were 2.62%, 62.15%, 4.91% and 51.98%, respectively. These values are reproduced from the supplied report and have not been independently recalculated.

The reported data show differences among the extracts, with the methanolic extract displaying the highest listed inhibition at both 500 and 4 µg/mL. However, the source table does not assign the individual values separately to *E. coli* and *S. aureus*. In addition, several essential details of the resazurin protocol, including

some volumes and final concentrations, were missing from the supplied report. These results should therefore be treated as descriptive observations rather than definitive evidence of organism-specific antibacterial potency.

The accompanying narrative also reports MIC values for samples designated Kb1, Kb2, Kb3 and Kb4. According to that narrative, Kb1 and Kb3 had reported MIC values of 0.8 µg/mL against *S. aureus* and 125 µg/mL against *E. coli*. Kb2 and Kb4 were reported to have MIC values of 125 µg/mL against *S. aureus* and 250 µg/mL against *E. coli*. However, the relationship between these Kb-labelled samples and the four solvent extracts described in the methods has not been established. These MIC values must therefore remain separate from the solvent-extract results until the sample identities and original microdilution records are confirmed.

5.3 Integrated Interpretation of Antioxidant and Antibacterial Findings

The findings indicate that Glycine max residue extracts exhibited solvent-dependent differences in DPPH radical-scavenging activity and reported bacterial growth inhibition. The n-hexane extract showed the highest reported antioxidant activity (approximately 82–83%) at 50 µg/mL, whereas the methanolic extract showed the highest reported growth-inhibition value at 500 µg/mL (approximately 89.71%). These findings suggest that different extracts may possess distinct biological properties. However, the antioxidant and antibacterial results require further verification because raw absorbance data, numerical inhibition-zone measurements and complete organism-specific MIC records were unavailable.

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