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Phytochemical Profiling and Multitarget Bioactivities of *Sophora flavescens* Leaf Extracts: Antibacterial, Antifungal, Antioxidant, and Hyaluronidase Inhibition

Research Article

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Abstract

Sophora flavescens (Fabaceae), known as Kushen in Traditional Chinese Medicine (TCM), has been used for millennia to treat fevers, dysentery, eczema, and inflammatory disorders. Phytochemical screening of five solvent extracts (hexane, ethyl acetate, acetone, methanol, and aqueous) revealed a distinct polarity-dependent extraction pattern, with carbohydrates, proteins, flavonoids, saponins, tannins, and glycosides exhibiting differential solubility, while alkaloids were predominantly recovered in organic phases. Quantitative estimation demonstrated that SF-EAE (ethyl acetate extract) possessed the highest total phenolic content (17.4 mg GAE/g DW) and total flavonoid content (11.2 mg QE/g DW), followed by SF-ME (methanol extract) with 14.7 mg GAE/g DW and 9.1 mg QE/g DW, and SF-AqE (aqueous extract) with 9.5mg GAE/g DW and 6.8 mg QE/g DW, while SF-HE (hexane extract) and SF-AE (acetone extract) showed complete absence of flavonoids. Antibacterial evaluation revealed concentration-dependent activity, with SF-HE exhibiting remarkable efficacy against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (up to 14.0 mm zone of inhibition at 200 µg/mL) despite containing no phenolics or flavonoids, indicating the significant role of alkaloids against Gram-negative pathogens. In contrast, SF-EAE and SF-ME demonstrated superior activity against *Bacillus subtilis*, correlating strongly with their phenolic and flavonoid loads. SF-EAE and SF-ME exhibited the most potent antifungal activity (up to 14.5 mm at 200 µg/mL), with SF-AqE showing preferential activity against *Aspergillus niger* (9.6 mm) compared to *Candida albicans* (7.6 mm), suggesting selective efficacy of polar glycosides against filamentous fungi through hyphal growth interference. The DPPH radical scavenging activity was highest for SF-ME (83.19% at 100 µg/mL), whereas ABTS assay revealed SF-EAE as the most potent (72.35%), with SF-HE showing substantially enhanced ABTS activity (70.13%) compared to DPPH (60.24%). Hyaluronidase inhibition assay demonstrated SF-EAE as the most effective inhibitor (52.42% at 250 µg/mL), followed by SF-ME (49.35%), with SF-HE retaining considerable activity (32.34%). S. *flavescens* possesses multitarget therapeutic potential through synergistic action of diverse phytochemical classes flavonoid aglycones, phenolic glycosides, and alkaloids each contributing uniquely to antibacterial, antifungal, antioxidant, and enzyme inhibitory activities, thereby validating its traditional use in TCM. The study underscores the critical role of solvent selection in optimizing bioactive compound recovery, with ethyl acetate emerging as the solvent of choice for flavonoid aglycones, methanol providing comprehensive broad-spectrum extraction, and hexane selectively recovering lipophilic alkaloids with distinct bioactivity profiles against Gram-negative bacteria.

Keywords: *Senna sophera* L; Foliar micromorphology; anatomy; xylem element; microchemical study.

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Introduction

Sophora flavescens Aiton, commonly known as Kushen in Chinese, is a perennial shrub belonging to the family Fabaceae (Leguminosae) and the genus Sophora [1]. The genus Sophora is widespread throughout Asia, Oceania, and the Pacific Islands, comprising approximately 52 species, 19 varieties, and 7 forms. The plant was first described by William Aiton in Hortus Kewensis published in 1789 [2]. *S. flavescens* is native to a wide area of East Asia, including China, Mongolia, Taiwan, Japan, Korea, India, Russia (Siberia), and some European countries. The plant is a deciduous semi-shrub or herbaceous subshrub, typically growing to a height of 0.5–3 m [3]. The stem is erect, green or yellow-brown, with longitudinal grooves, sparsely covered with soft hairs when young, and becoming glabrous with age. The root is cylindrical, externally yellowish-white to gray-brown, and can reach up to 1 meter in length. The leaves are odd-pinnate compound, usually 20-25 cm long, with 13-36 leaflets that are elliptic, ovate, or lanceolate, measuring 3–6 cm in length and 1.2-2 cm in width. The plant produces terminal racemes measuring 15–25 cm, bearing numerous white, pale yellow, or purple-red flowers that bloom from June to August. The fruit is a legume pod, 5-10 cm long, slightly constricted between seeds, containing 1-5 seeds that are dark reddish-brown or purplish-brown [4,5].

S. flavescens has been an important species in traditional medicine since the Qin and Han dynasties in China. It has been used in China, Japan, Mongolia, India, and Korea for thousands of years as a highly traditional herbal medicine. The dried root of *S. flavescens*, known as Radix Sophorae Flavescentis, is the primary medicinal part and is recorded in the Compendium of Materia Medica (Bencao Gangmu) [6,7,8]. Phytochemical investigations have revealed that S. flavescens contains a diverse array of bioactive constituents. To date, more than 200 compounds have been isolated from this species. The major phytoconstituents are alkaloids and flavonoids, which are considered the main pharmacologically active components. Alkaloids constitute approximately 3.3% of the root composition, while flavonoids account for about 1.5%. More than 71 alkaloids have been isolated, among which quinolizidine alkaloids—particularly matrine, oxymatrine, sophoridine, sophocarpine, N-oxysophocarpine, d-allomatrine, d-isomatrine, d-sophoranol, and sophoranol N-oxide—are the most abundant and pharmacologically significant [9,10,11,12]. The flavonoid fraction includes compounds such as kushenol A, kurarinone, maackiain, trifolirhizin, sophoraflavanone G, and various prenylated flavonoids. Other chemical constituents present include alkylxanthones, quinones (such as kushequinone A), triterpene glycosides (including soyasaponin I), fatty acids, essential oils, phenylpropanoids, and sterols. Recent metabolomic studies have identified 227 flavonoids and 55 alkaloids across five different tissues (roots, stems, leaves, flowers, and pods) of S. flavescens, highlighting the chemical complexity and tissue-specific distribution of these bioactive compounds [13,14,15].

The diverse phytochemical composition of *S. flavescens* is responsible for its wide range of pharmacological activities. Modern pharmacological studies have demonstrated that extracts and pure compounds from *S. flavescens* exhibit potent antitumor activities against various cancer cell lines, including lung, breast, liver, ovarian, and prostate cancers. The major mechanisms of action include the generation of reactive oxygen species, induction of apoptosis, and modulation of key signaling pathways such as MAPK, PI3K/AKT, NF-κB, and JAK2/STAT3 [16,17]. The plant also exhibits significant antimicrobial properties, showing activity against a broad spectrum of bacteria and fungi [18,19]. Anti-inflammatory and antioxidant activities have been extensively documented, with compounds such as matrine, oxymatrine, kushenol C, and various prenylated flavonoids playing key roles [18,19,20,21,22]. Furthermore, *S. flavescens* has demonstrated antiviral [23], antipyretic [24], hepatoprotective [25,26], neuroprotective [27,28], immunomodulatory [29,30], antiarrhythmic [31,32,33], and antiallergic effects [34,35].

Recent studies have demonstrated that the aboveground parts, including leaves, contain a rich diversity of flavonoids and alkaloids, with 156 differentially accumulated metabolites identified in leaves compared to roots. The present study was undertaken to systematically evaluate the phytochemical profile and biological activities of *S. flavescens* leaf extracts obtained using five different solvents of varying polarity: hexane, ethyl acetate, acetone, methanol, and distilled water. The crude extracts were subjected to preliminary phytochemical screening to identify the presence of various bioactive constituents [36-44]. Quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) was performed using standard colorimetric methods [41,42]. The antimicrobial activity was evaluated against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), and fungal strains (*Candida albicans* and *Aspergillus niger*) using the disc diffusion method [45,46]. The antioxidant potential was assessed through three different *in-vitro* assays: DPPH, H_2O_2, and ABTS radical scavenging assays [47-49]. The hyaluronidase inhibitory activity of the extracts was assessed to evaluate their potential in modulating extracellular matrix degradation [50,51]. This comprehensive investigation aims to provide a scientific basis for the potential utilization of *S. flavescens* leaves as a source of bioactive compounds with therapeutic applications and to contribute to the rational development and utilization of the aboveground parts of this important medicinal plant.

Materials and Methods

Plant Material Collection and Extraction

Fresh and healthy leaves of *S. flavescens* were collected, thoroughly washed under running tap water to remove debris, and shade-dried at room temperature. The plant species was taxonomically authenticated

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by a botanist, and a voucher specimen (ZD-SF-01) was deposited at the institutional herbarium for future reference. The dried leaves were ground into a coarse powder using a mechanical grinder. Five separate 100 g portions of the dried powder were independently extracted with each of the following five solvents (i.e., extractions were performed in parallel for each solvent, not sequentially on the same batch): Hexane (non-polar), Ethyl Acetate (medium-polar), Acetone (polar aprotic), Methanol (polar protic), and Distilled Water (highly polar). For each solvent batch, the powder was initially macerated in 500 mL of the respective solvent on an orbital shaker for 48-72 hours at room temperature. After this maceration period, each mixture was filtered through Whatman No. 1 filter paper. The filtrate (maceration extract) was collected, and the residual solid marc was then subjected to Soxhlet extraction using fresh solvent (500 mL of the same respective solvent) for 6-8 hours, continuing until the solvent in the siphon tube became clear. The Soxhlet extract obtained was then pooled with the previously collected maceration filtrate for each solvent. The pooled extracts were concentrated under reduced pressure using a rotary evaporator. The concentrated extracts were then dried in a water bath and stored in airtight containers at 4°C until further analysis [52]. The extraction yield for each solvent, calculated as (weight of dried extract / weight of dried powder) × 100, was recorded. The extracts were named as *S. flavescens* hexane extract (SF-HE), *S. flavescens* ethyl acetate extract (SF-EAE), *S. flavescens* acetone extract (SF-AE), *S. flavescens* methanol extract (SF-ME), and *S. flavescens* aqueous extract (SF-AqE).

Phytochemical Screening

The obtained crude extracts were subjected to preliminary qualitative phytochemical screening to identify the presence of various bioactive constituents using standard protocols. The presence of carbohydrates was detected using the molisch test, where a few drops of α-naphthol solution were added to the extract, followed by the careful addition of concentrated sulfuric acid along the side of the test tube, with the formation of a violet ring at the interface indicating a positive result [36]. Proteins were confirmed using the biuret method, wherein an equal volume of 5% sodium hydroxide solution and a few drops of 1% copper sulfate solution were added to the extract, and the formation of a violet or pink color indicated the presence of proteins [37]. The presence of amino acids was assessed using the ninhydrin test, where a few drops of ninhydrin solution were added to the extract and heated in a boiling water bath; the development of a purple or blue color confirmed their presence [38]. For the detection of cholesterol and other sterols, the liebermann-burchard reaction was performed. The extract was dissolved in chloroform, and after adding a few drops of acetic anhydride and concentrated sulfuric acid, a color change from red to blue or green confirmed the presence of sterols [39]. Alkaloids were screened using standard alkaloid-precipitating reagents, where the formation of characteristic reddish-brown or orange-red precipitates indicated their presence [40]. The presence and quantification of total phenols were estimated colorimetrically using Singleton's method [41], while flavonoids were determined using the aluminum chloride colorimetric assay [42]. Tannins were estimated using the ferric chloride ($FeCl_3$) assay [43] and various tests were performed for the detection of glycosides and saponins, including the keller–Keilani test (for cardiac glycosides) and borntrager's test (for anthraquinone glycosides). Saponin glycosides were identified using the foam test, and all tests were interpreted according to standard protocols [44].

Determination of Total Phenolic Content (TPC)

TPC of the five *S. flavescens* leaf extracts was determined using the folin-ciocalteu (F-C) colorimetric method as described by Singleton et al. (1965). Gallic acid was used as the standard reference compound. A stock solution of gallic acid was prepared at a concentration of 1 mg/mL in methanol, from which working standard solutions of varying concentrations (20–100 µg/mL) were prepared by serial dilution. For the assay, 0.5 mL of each extract solution (prepared at a concentration of 100 µg/mL in methanol) was mixed with 2.5 mL of F-C reagent (diluted 1:10 with distilled water) in a test tube and thoroughly vortexed. The mixture was allowed to stand at room temperature for 5 minutes. Subsequently, 2 mL of 7% sodium carbonate (Na_2CO_3) solution was added, and the final volume was made up to 10 mL with distilled water. The reaction mixture was incubated in the dark at room temperature for 30-90 minutes to allow for complete color development. The absorbance was measured spectrophotometrically at 760 nm (or 765 nm) against a reagent blank prepared with distilled water instead of the extract. A standard calibration curve was constructed by plotting the absorbance of gallic acid standards against their respective concentrations. TPC was calculated from the calibration curve equation and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g) [41].

Determination of Total Flavonoid Content (TFC)

TFC of the five *S. flavescens* leaf extracts was determined using the aluminum chloride ($AlCl_3$) colorimetric method. A stock solution of the standard (quercetin) was prepared in methanol at a concentration of 1 mg/mL, and working standard solutions of varying concentrations (10–100 µg/mL) were prepared by serial dilution. For the assay, 0.5 mL of each extract solution (prepared at a concentration of 100 µg/mL in methanol) was mixed with 2 mL of distilled water in a test tube. To this mixture, 0.15 mL of 5% sodium nitrite ($NaNO_2$) solution was added and allowed to stand for 6 minutes. Then, 0.15 mL of 10% $AlCl_3$ solution was added, and the mixture was allowed to stand for another 6 minutes. Finally, 2 mL of 1 M sodium hydroxide (NaOH) solution was added. The final volume was made up to 5 mL with distilled water, and the mixture was thoroughly vortexed. After incubation at room temperature for 15–30 minutes, the absorbance was measured

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spectrophotometrically at 510 nm (or 415–430 nm) against a reagent blank prepared with distilled water instead of the extract. A standard calibration curve was constructed by plotting the absorbance of the standard solutions against their respective concentrations. TFC was calculated from the calibration curve equation and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g). All experiments were performed in triplicate [42].

Evaluation of Antibacterial and Antifungal Activity

The antimicrobial activity of the five S. flavescens leaf extracts was evaluated against a specific panel of pathogenic microorganisms using the agar disc diffusion method [45,46]. The test organisms included Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus), Gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli), and fungal strains (Candida albicans and Aspergillus niger). The bacterial and fungal strains were subcultured on nutrient agar (NA) and potato dextrose agar (PDA), respectively, and incubated overnight. A bacterial suspension was prepared in sterile saline and adjusted to a 0.5 McFarland standard to achieve a standard inoculum size. NA for bacteria and PDA for fungi were poured into sterile Petri dishes and allowed to solidify. The microbial suspensions were evenly spread over the agar surface using a sterile cotton swab. Whatman No. 1 filter paper discs (6 mm in diameter) were impregnated with various concentrations of the five extracts: 50 µg/mL, 100 µg/mL, 150 µg/mL, and 200 µg/mL. The impregnated discs were air-dried to evaporate the solvent and placed aseptically onto the surface of the inoculated agar plates. After incubation at 37 °C for 24 h (bacteria) or at 25 °C for 48–72 h (fungi), the resulting zones of inhibited microbial growth were measured. The total diameter of each clear inhibition zone, inclusive of the 6 mm disc, was recorded in millimeters using a calibrated Vernier caliper. A standard antibiotic (for bacteria) and a standard antifungal drug (for fungi) were used as positive controls, while the respective solvents (e.g., distilled water, methanol, hexane) served as negative controls [45,46].

DPPH Free Radical Scavenging Assay

The ability of the extracts to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined. A DPPH stock solution (1 mM/L) was prepared by dissolving 8 mg of DPPH in 200 mL of methanol. Different concentrations of the five *S. flavescens* leaf extracts (20, 40, 60, 80, and 100 µg/mL) were dissolved in 1 mL of methanol separately in each test tube. To this solution, 2 mL of the DPPH stock solution was added, and the mixture was kept in the dark for 45 minutes at room temperature. After incubation, the absorbance values were recorded at 517 nm. Ascorbic acid was used as a positive control, and a blank was prepared using methanol instead of the extract. DPPH scavenging activity was calculated using the equation % Scavenging = [(Control Absorbance – Test Compound Absorbance) / Control Absorbance] × 100. Where Control Absorbance = Absorbance of DPPH solution without extract (methanol blank). Test Compound Absorbance = Absorbance of DPPH solution with the extract [47,48].

ABTS Radical Scavenging Assay

The ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity of the extracts was evaluated according to the standard protocol. The $ABTS^{\bullet+}$ was generated by mixing an ABTS stock solution (7 mM) with potassium persulfate (2.45 mM). The mixture was incubated in the dark for 12–16 hours at room temperature to obtain a stable absorbance value. The ABTS radical cation solution was then diluted with 70% ethanol to achieve an absorbance of 0.700 ± 0.02 at 734 nm, which served as the ABTS radical working solution. For the assay, 0.9 mL of this working solution was added to 0.1 mL of different concentrations (20, 40, 60, 80, and 100 µg/mL) of the five *S. flavescens* leaf extracts. The mixture was shaken well for 45 seconds and incubated in the dark for 15 minutes at room temperature. The absorbance was then measured at 734 nm. Ascorbic acid was used as a positive control, and a blank was prepared using 70% ethanol instead of the extract. The ABTS radical scavenging activity (RSA) was calculated using the following formula. % ABTS-RSA = [(Ac – At) / Ac] × 100. Where: Ac = Absorbance of the control (ABTS radical working solution without extract). At = Absorbance of the test sample (ABTS radical working solution with extract) [49].

Hyaluronidase Inhibitory Activity

The hyaluronidase inhibitory potential of the five *S. flavescens* leaf extracts was evaluated using the colorimetric method described by Morgan and Elson [50,51]. The assay relies on the ability of test compounds to inhibit the enzymatic degradation of sodium hyaluronate by hyaluronidase, with the remaining undegraded substrate quantified chromogenically. In brief, 0.05 mL of calcium chloride solution (2.5 mol/L) was pipetted into four separate 10-mL test tubes designated as A, B, C, and D. To tubes A and C, 0.25 mL of acetic acid buffer was added, while tubes B and D received 0.25 mL of hyaluronidase enzyme solution (500 U/mL). The four tubes were incubated for 20 minutes at 37 °C to activate the enzyme. Following this initial incubation, 0.25 mL of each extract (prepared at appropriate concentrations in the respective solvents) was introduced into tubes A and B, whereas 0.25 mL of distilled water was added to tubes C and D to serve as controls. The tubes were then incubated once again for 20 minutes at 37 °C to allow for enzyme-inhibitor interaction. Subsequently, 0.25 mL of sodium hyaluronate (the enzyme substrate) was added to tubes B and D, and 0.25 mL of acetate buffer was added to tubes A and C. All four tubes were then incubated at 37 °C for 40 minutes to facilitate the enzymatic reaction. To stop the reaction and develop the chromophore, 0.05 mL of sodium hydroxide (NaOH), 0.5 mL of acetylacetone, and 0.25 mL of distilled water were added to each tube. The tubes were then placed in a boiling water bath for 30 minutes,

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Table 1: Phytochemical Screening of *S. flavescens* extracts.

Bioactive compounds	*S. flavescens* extracts				
	SF-HE	SF-EAE	SF-AE	SF-ME	SF-AqE
Carbohydrates	-	+	+	+	+
Proteins	-	+	-	+	+
Lipids	+	-	+	-	-
Alkaloids	+	+	+	+	-
Flavonoids	-	+	-	+	+
Saponins	-	-	-	+	+
Tannins	-	+	-	+	+
Glycosides	-	-	+	+	+

after which they were immediately cooled in an ice bath. Finally, 0.5 mL of p-dimethylaminobenzaldehyde (DMAB) chromogenic reagent solution was added to all four tubes, and the mixture was allowed to stand at room temperature for 30 minutes to achieve complete color development. The absorbance of each reaction mixture was recorded spectrophotometrically at 530 nm. The percentage of hyaluronidase inhibition (HI %) was calculated using the following formula: HI (%) = [(A − B) − (C − D)] / (A − B) × 100. Where: A = Absorbance of the control reaction mixture (acetate buffer + sample + acetate buffer). B = Absorbance of the test reaction mixture (hyaluronidase + sample + sodium hyaluronate). C = Absorbance of the reagent blank (acetate buffer + distilled water + acetate buffer). D = Absorbance of the enzyme control (hyaluronidase + distilled water + sodium hyaluronate). All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD) [50,51].

Statistical Analysis

All experiments were conducted with a minimum of three independent biological replicates, and each replicate was performed in technical triplicate (n = 3). The data are presented as the arithmetic mean ± standard deviation (SD). Statistical comparisons among multiple treatment groups and concentrations were performed using a one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for pairwise comparisons where applicable. For comparisons between two groups (e.g., extract versus control), Student's unpaired t-test was employed. All statistical analyses were executed using SPSS Statistics software (Version 22.0, IBM Corp., Armonk, NY, USA). A probability value (p) of less than 0.050 was considered statistically significant for all analyses.

Results and Discussion

Phytochemical screening of various extracts of S. flavescens

The screening data for *S. flavescens* reveals a distinct polarity-dependent extraction pattern, clearly demonstrating how solvent selection governs the phytochemical profile **(Table 1).** The extraction yields, calculated as (weight of dried extract / weight of dried powder) × 100, varied considerably across solvents: SF-HE (3.5 g/100 g DW), SF-AE (4.2 g/100 g DW), SF-EAE (4.6 g/100 g DW), SF-AqE (5.1 g/100 g DW), and SF-ME (5.6 g/100 g DW). This yield hierarchy (ME > AqE > EAE > AE > HE) broadly follows increasing solvent polarity, with methanol and water recovering the highest mass of extractable materials, consistent with their ability to dissolve a wide spectrum of polar to moderately polar phytochemicals, while the lower yields of hexane and acetone reflect their more selective extraction of specific compound classes.

Specifically, screening revealed that carbohydrates are present in SF-EAE, SF-AE, SF-ME, and SF-AqE, but are completely absent from the non-polar SF-HE. Proteins were detected in SF-EAE, SF-ME, and SF-AqE, yet were notably absent in both SF-HE and SF-AE. Conversely, lipids were exclusively localized to SF-HE and SF-AE, confirming their solubility in organic, non-aqueous phases while remaining completely absent from the more polar methanol and aqueous extracts. Regarding alkaloids, screening revealed their presence in SF-HE, SF-EAE, SF-AE, and SF-ME, with their complete absence from SF-AqE suggesting they exist primarily as free bases rather than water-soluble salts in this plant. For flavonoids, positivity was observed in SF-EAE, SF-ME, and SF-AqE, while SF-HE and SF-AE tested negative, indicating that pure acetone is ineffective for their recovery despite aqueous acetone being widely used for total flavonoids. Similarly, tannins mirrored this pattern, being present in SF-EAE, SF-ME, and SF-AqE, but absent from hexane and pure acetone extracts, which aligns with their known hydrogen-bonding requirements. Notably, saponins were restricted exclusively to SF-ME and SF-AqE, highlighting their strict requirement for highly polar protic solvents and their complete exclusion from organic phases like hexane, ethyl acetate, and acetone. Finally, glycosides were detected in SF-AE, SF-ME, and SF-AqE, but were absent from SF-HE and SF-EAE, confirming that intact sugar-conjugated compounds demand aqueous or highly polar conditions for effective dissolution.

Examining these profiles on a per-extract basis further reinforces these trends. The SF-HE exclusively yielded lipids and alkaloids, confirming its non-polar nature by selectively dissolving fatty constituents and free-base alkaloids while leaving all polar compounds unextracted. In contrast, SF-EAE showed a moderately polar profile with positive results for alkaloids, flavonoids, tannins, and surprisingly, carbohydrates and proteins, yet it lacked saponins and

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glycosides, indicating that ethyl acetate effectively extracts aglycones and some amphiphilic proteins but fails to recover intact sugar-rich conjugates. The SF-AE presented a unique pattern, containing lipids, alkaloids, and glycosides, but testing negative for flavonoids, saponins, and tannins; this suggests that pure acetone, without aqueous modification, may precipitate or inadequately solvate these polyphenolic and triterpenoid compounds. Notably, SF-ME emerged as the most exhaustive extract, returning positive results for every tested compound except lipids, cementing its status as the gold standard for broad-spectrum recovery of polar to moderately polar bioactives, including carbohydrates, proteins, flavonoids, saponins, tannins, and glycosides. Meanwhile, SF-AqE effectively extracted all polar moieties, carbohydrates, proteins, flavonoids, saponins, tannins, and glycosides, but completely lacked alkaloids and lipids, implying that the alkaloids in this plant exist predominantly as non-polar free bases rather than water soluble salts.

Quantitative estimation of TPC in *S. flavescens* extracts

Quantitative estimation of TPC in *S. flavescens* extracts, expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW), revealed a distinct solvent-dependent hierarchy **(Table 2).** As anticipated, SF-HE showed a complete absence of phenolics, confirming that non-polar hexane cannot solubilize these polar aromatic compounds. Remarkably, SF-EAE exhibited the highest TPC at 17.4 mg GAE/g DW, surpassing all other extracts—this finding aligns with ethyl acetate's reputation as the preferred solvent for phenolic aglycones and low-molecular-weight polyphenols, which partition effectively into its moderate polarity. The methanol extract followed closely with 14.7 mg GAE/g DW, demonstrating its broad-spectrum efficiency, though its slightly lower value than EAE suggests that a substantial portion of phenolics in this plant exist as aglycones rather than glycosides, favoring ethyl acetate over methanol. The aqueous extract recorded 9.5 mg GAE/g DW, effectively capturing polar phenolic glycosides, yet its moderate yield indicates that water alone fails to release bound or cell wall-associated phenolics. SF-AE displayed the lowest quantifiable value at just 4.1 mg GAE/g DW, corroborating the qualitative screening where acetone tested negative for flavonoids and tannins—pure acetone, lacking sufficient water content, inadequately solvates polyphenolic structures and likely precipitates them during extraction. The overall TPC ranking (EAE > ME > AqE > AE > HE) underscores that moderately polar solvents, particularly ethyl acetate and methanol, are most effective for phenolic recovery from *S. flavescens*. These quantitative data perfectly complement the qualitative phytochemical screening, reinforcing the critical role of solvent polarity and aqueous modification in determining phenolic extractability.

Table 2: TPC and TFC for all five extracts of *S. Flavescens*.

Extract	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
SF-HE	0 (Absent)	0 (Absent)
SF-EAE	17.4	11.2
SF-AE	4.1	0 (Absent)
SF-ME	14.7	9.1
SF-AqE	9.5	6.8

Table 3: Antibacterial activity of *S. flavescens* extracts against both Gram+ve and Gram-ve bacteria.

Bacteria	ug/mL	SF-AqE	SF-ME	SF-AE	SF-EAE	SF-HE
B. subtilis	50	-	3.4 ± 0.20	2.0 ± 0.14	3.24 ± 0.15	1.9 ± 0.60
	100	2.3 ± 0.11	5.8 ± 0.17	3.4 ± 0.22	6.22 ± 0.24	3.1 ± 0.15
	150	3.2 ± 0.15	8.1 ± 0.21	5.5 ± 0.35	9.24 ± 0.36	5.7 ± 0.98
	200	6.6± 0.18	11.2 ± 0.50	7.2 ± 0.42	11.5 ±0.62	8.31 ± 0.02
S. aureus	50	-	4.2 ± 0.15	-	4.2 ± 0.24	3.7 ± 0.60
	100	1.5 ± 0.35	6.1 ± 0.24	2.2 ± 0.1	6.4 ± 0.35	8.1 ± 0.20
	150	2.5± 0.00	8.5 ±0.50	3.6 ± 0.15	9.1 ± 0.44	9.7 ± 0.56
	200	3.0 ±0.19	11.4 ±0.20	6.2 ± 0.40	11.8 ± 1.3	12.0 ± 0.42
E. coli	50	-	3.2 ± 0.40	1.2 ± 0.00	3.62 ± 0.25	6.3 ± 0.11
	100	1.7 ± 0.50	4.8 ± 0.20	3.6 ± 0.28	6.14 ± 0.28	8.0 ± 0.54
	150	2.3 ± 0.11	6.2 ± 0.32	5.8 ± 0.36	9.12 ± 0.24	9.2 ± 0.00
	200	3.5 ± 0.00	9.5 ± 0.50	7.6 ± 0.44	11.2 ±0.52	14.0 ± 0.15
P. aeruginosa	50	-	2.8 ± 0.24	-	3.2 ± 0.14	2.3 ± 0.51
	100	2.6 ± 0.15	4.1 ± 0.26	1.8 ± 0.15	5.8 ± 0.32	6.9 ± 0.03
	150	3.2 ± 0.11	6.5 ±0.30	3.9 ± 0.20	6.1 ± 0.24	10 ± 0.20
	200	4.0 ± 0.11	8.4 ±0.32	5.2 ± 0.41	9.8 ± 1.31	11 ± 0.38

Quantitative estimation of TFC in *S. flavescens* extracts

Quantitative estimation of TFC in *S. flavescens* extracts, expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW), revealed a distinct polarity-driven distribution that perfectly corroborates the qualitative screening data **(Table 2).** As expected, SF-HE showed a complete absence of flavonoids, confirming that non-polar hexane cannot dissolve these moderately polar compounds. Similarly, SF-AE also exhibited zero flavonoid content, aligning with the qualitative screening where acetone tested negative, pure acetone, devoid of sufficient water content, fails to solvate flavonoid structures and likely precipitates them during extraction. Among the positive extracts, SF-EAE recorded the highest TFC at 11.2 mg QE/g DW, demonstrating its exceptional affinity for flavonoid aglycones, which are moderately polar and partition efficiently into this solvent. The SF-ME followed with 9.1 mg QE/g DW, indicating excellent broad-spectrum recovery, yet its lower value compared to EAE suggests that flavonoids in *S. flavescens* exist predominantly as aglycones rather than

Citation: Yulong W, Yani L, PengFei L, Jing Y, Jing Y, et al. Phytochemical Profiling and Multitarget Bioactivities of Sophora flavescens Leaf Extracts: Antibacterial, Antifungal, Antioxidant, and Hyaluronidase Inhibition. J Plant Sci Res. 2026;13(2): 298.

glycosides, favouring ethyl acetate over methanol. SF-AqE displayed the lowest quantifiable content at just 6.8 mg QE/g DW, effectively capturing flavonoid glycosides but missing the aglycone fraction entirely, resulting in a reduced overall yield. The overall TFC ranking (EAE > ME > AqE > AE = HE) demonstrates that ethyl acetate is the most effective solvent for flavonoid recovery from this plant, while methanol remains a reliable alternative for comprehensive profiling. These quantitative findings are entirely consistent with the polarity principles governing flavonoid solubility and underscore the critical importance of selecting appropriate solvents based on the target flavonoid forms aglycones versus glycosides for optimal extraction efficiency in future pharmacological investigations.

Antibacterial activity of *S. flavescens* extracts

The antibacterial activity of *S. flavescens* extracts against *Bacillus subtilis*, measured as zone of inhibition (mm), demonstrated a clear concentration-dependent response across all five solvent extracts **(Table 3)**. Among all extracts, SF-EAE consistently exhibited the highest activity at every concentration, with inhibition zones ranging from 3.24 mm at 50 µg/mL to 11.5 mm at 200 µg/mL, closely followed by SF-ME which showed 3.4 mm to 11.2 mm over the same concentration range; this superior performance of EAE and ME showed a positive association with their elevated total phenolic and flavonoid contents (17.4 mg GAE/g and 11.2 mg QE/g, respectively). The SF-AqE showed moderate activity, increasing from 2.3 mm to 6.6 mm, while SF-AE and SF-HE displayed the weakest inhibitory effects, with hexane consistently yielding the lowest zones (1.9 mm to 8.31 mm), which is consistent with its absence of phenolics and flavonoids. The ranking of antibacterial efficacy (EAE ≥ ME > AqE > AE > HE) closely paralleled the TPC and TFC rankings.

The antibacterial activity of *S. flavescens* extracts against *S. aureus* exhibited a marked concentration-dependent increase across all active extracts. SF-HE displayed substantial activity, with inhibition zones increasing from 3.7 mm at 50 µg/mL to an impressive 12.0 mm at 200 µg/mL, surpassing even SF-EAE (11.8 mm) at the highest concentration. This is particularly striking given that SF-HE is completely devoid of phenolics and flavonoids, suggesting a potential role for its alkaloid and lipophilic constituents in the activity against *S. aureus*. In contrast, SF-AqE, which lacks alkaloids entirely, exhibited the weakest activity (just 3.0 mm at 200 µg/mL) and showed no inhibition at 50 µg/mL, further supporting the hypothesis that alkaloids may contribute substantially to anti-staphylococcal activity. Both SF-EAE and SF-ME also demonstrated excellent activity, reaching 11.8 mm and 11.4 mm respectively at 200 µg/mL, which is consistent with their high phenolic and flavonoid loads. Meanwhile, SF-AE displayed no inhibition at 50 µg/mL but showed moderate activity at higher doses (6.2 mm at 200 µg/mL), while SF-AqE only became active from 100 µg/mL upwards (1.5 mm). The superior performance of SF-HE against *S. aureus*—unlike its weaker action against *B. subtilis* highlights pathogen-specific susceptibility profiles and raises the possibility that the alkaloid-rich hexane fraction may contain selective bioactive principles targeting this particular strain [18,19,50].

Remarkably, SF-HE emerged as the most potent inhibitor of *E. coli*, recording the highest zone of inhibition at 200 µg/mL (14.0 mm), surpassing even the ethyl acetate extract (11.2 mm) and methanol extract (9.5 mm) at the same concentration; this parallels its superior performance against *S. aureus* and starkly contrasts with its weak activity against *Bacillus subtilis*, raising the possibility that the lipophilic alkaloids unique to SF-HE may confer selective efficacy against *E. coli* and *S. aureus* but not *B. subtilis*. The ethyl acetate extract (SF-EAE) displayed robust activity ranging from 3.62 mm to 11.2 mm, which showed a positive trend consistent with its high phenolic and flavonoid content (17.4 mg GAE/g and 11.2 mg QE/g), suggesting, though not proving that these polar aglycones may contribute to anti-*E. coli* effects. SF-ME followed with inhibition zones progressing from 3.2 mm to 9.5 mm, while SF-AE showed moderate activity (1.2 mm to 7.6 mm), both broadly aligning with their respective phytochemical profiles. Critically, SF-AqE, which completely lacks alkaloids, was the least effective against *E. coli*, showing no inhibition at 50 µg/mL and reaching only 3.5 mm at 200 µg/mL; this observation lends circumstantial support to the hypothesis that alkaloids, rather than polar phenolics alone, may be the predominant bioactive class against this Gram-negative strain. Interestingly, SF-HE demonstrated significant activity even at the lowest concentration (6.3 mm at 50 µg/mL), which may indicate rapid action of its bioactive constituents, though this interpretation remains speculative without time-kill kinetics [18,19,45,46,50].

The antibacterial activity of *S. flavescens* extracts against the opportunistic Gram-negative pathogen *P. aeruginosa* demonstrated a clear concentration-dependent escalation, with patterns broadly mirroring those observed against *E. coli* and *S. aureus*. Remarkably, SF-HE once again exhibited the most potent activity at higher concentrations, escalating from 2.3 mm at 50 µg/mL to an impressive 11.0 mm at 200 µg/mL, surpassing SF-EAE (9.8 mm) at the highest dose and further highlighting the activity associated with its alkaloid-rich, non-phenolic profile. SF-EAE showed robust and consistent activity ranging from 3.2 mm to 9.8 mm, paralleling its superior phenolic and flavonoid content (17.4 mg GAE/g and 11.2 mg QE/g), while SF-ME followed with moderate inhibition zones progressing from 2.8 mm to 8.4 mm. Notably, SF-AE and SF-AqE exhibited the weakest effects, with SF-AqE completely inactive at 50 µg/mL and reaching only 4.0 mm at 200 µg/mL; this pattern indirectly supports—but does not confirm—a hypothesized role for alkaloids (absent in AqE and poorly extracted by pure acetone) in countering *P. aeruginosa*. Interestingly, SF-HE displayed a dramatic surge in activity between 100 and 150 µg/mL (6.9 mm to 10.0 mm), suggesting

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a possible threshold concentration effect for its lipophilic bioactives, whereas SF-EAE showed a more gradual increase across the same range [18,19,45,46,50].

The observed concentration-dependent antibacterial effects, combined with the differential activity patterns among extracts, provide a basis for proposing several plausible mechanisms of action though these remain speculative without direct molecular validation (Figure 1). Drawing upon established literature on similar phytochemical classes, the antibacterial action is hypothesized to involve: (i) disruption of cell wall synthesis, potentially leading to structural weakening; (ii) increased membrane permeability, which may cause leakage of cellular contents; (iii) interference with bacterial proteins and enzymes, possibly impairing critical biochemical pathways; (iv) inhibition of nucleic acid synthesis, which could block replication and cell division; and (v) potential anti-quorum sensing and biofilm inhibition effects, which might reduce bacterial virulence and persistence. It is critically important to emphasize that the disc diffusion assay employed in this study only measures net growth inhibition (zone of clearance); it does not distinguish between bactericidal, bacteriostatic, or specific target-based effects. Therefore, while these proposed mechanisms are consistent with the observed bioactivity patterns and the known properties of phenolic/flavonoid and alkaloid compounds, they remain hypothetical. Direct experimental validation—such as electron microscopy for membrane integrity, fluorescence-based membrane permeability assays, enzyme inhibition kinetics, and nucleic acid intercalation studies—is required to confirm the exact modes of action. The differential activity profiles observed among extracts merely suggest that multiple compound classes (e.g., flavonoid aglycones in SF-EAE and lipophilic alkaloids in SF-HE) may operate through distinct, potentially complementary pathways; this interpretation warrants further bioassay-guided fractionation and target-specific investigations [18,19,45,46,50].

Antifungal activity of *S. flavescens* extracts

The antifungal activity of *S. flavescens* extracts against *Candida albicans* and *Aspergillus niger* demonstrated a clear concentration-dependent increase across all five solvent extracts (SF-AqE, SF-ME, SF-AE, SF-EAE, and SF-HE), with inhibition zones ranging from 2.1 mm to 14.5 mm over the 50–200 µg/mL concentration range. Against *C. albicans*, SF-EAE and SF-ME exhibited the most potent antifungal activity, with SF-EAE showing zones of inhibition from 7.21 mm at 50 µg/mL to 14.5 mm at 200 µg/mL, and SF-ME displaying comparable activity from 5.4 mm to 14.2 mm at the same concentrations (Figure 2). This superior performance was positively associated with their elevated total phenolic and flavonoid content, particularly the enrichment of flavonoid aglycones in SF-EAE (11.2 mg QE/g) and the broad-spectrum phenolic glycosides in SF-ME (14.7 mg GAE/g). This association raises the possibility—though it does not confirm—that these polyphenolic constituents may contribute to the observed anticandidal effects. However, the disc diffusion assay measures only net growth inhibition; the specific mechanisms (such as membrane disruption or enzyme inhibition) remain hypothetical without direct experimental validation. The SF-AE extract exhibited moderate activity against *C. albicans*, with inhibition zones progressing from 4.0 mm to 10.2 mm, which broadly aligned with its moderate phenolic content (4.1 mg GAE/g) despite the complete absence of flavonoids, tentatively suggesting that non-flavonoid phenolics such as phenolic acids may also play a role. SF-AqE and SF-HE displayed comparatively lower activity against *C. albicans*, with SF-AqE showing 2.8 mm to 7.6 mm and SF-HE showing 4.6 mm to 9.31 mm, suggesting that both polar phenolic glycosides and lipophilic alkaloids may contribute to anticandidal effects, albeit to a lesser extent than the flavonoid-rich ethyl acetate and methanol fractions in this assay system [18,19,45,46,50].

Against *Aspergillus niger*, a similar concentration-dependent trend was observed (Figure 3); however, the overall inhibition zones were notably smaller compared to *C. albicans*, **indicating that *A. niger* was relatively less susceptible** to the *S. flavescens* extracts under the tested conditions. SF-ME again demonstrated the strongest activity, with zones increasing from 5.4 mm to 11.8 mm at 200 µg/mL, followed closely by SF-EAE (4.3 mm to 10.2 mm) and SF-AE (3.0 mm to 9.2 mm), while SF-AqE showed moderate activity (3.8 mm to 9.6 mm). The SF-HE extract exhibited the weakest antifungal activity against *A. niger* (Figure 3) with inhibition zones ranging from just 2.1 mm to 6.22 mm, **raising the possibility** that lipophilic alkaloids may be comparatively less effective against this filamentous fungus than the polar phenolic-rich extracts. This reduced susceptibility of *A. niger* to SF-HE—despite its notable activity against Gram-negative bacteria—**highlights pathogen-specific susceptibility patterns and lends circumstantial support to the hypothesis** that fungal cell wall composition and membrane architecture may limit the access or efficacy of certain compound classes. Interestingly, SF-AqE demonstrated better activity against *A. niger* (9.6 mm at 200 µg/mL) than against *C. albicans* (7.6 mm), raising the tentative possibility that polar glycosides and other water-soluble constituents may exhibit relatively better efficacy against filamentous fungi —potentially through interference with hyphal growth and cell wall integrity, though this interpretation remains speculative without microscopy-based confirmation.

The observed concentration-dependent antifungal activity, together with the differential susceptibility of *C. albicans* and *A. niger* across solvent extracts, provides a basis for proposing several plausible mechanisms—though these remain speculative at this stage (Figure 4). Drawing upon established antifungal actions of plant polyphenols, flavonoids, and alkaloids in the literature, the following hypotheses are offered: (i) membrane disruption—phenolic and flavonoid compounds may interact with ergosterol-containing fungal

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membranes, potentially increasing permeability and causing leakage of cellular contents; (ii) enzyme inhibition—bioactive constituents might interfere with key fungal enzymes such as β-glucan synthase or ergosterol biosynthesis enzymes, thereby compromising cell wall and membrane synthesis; (iii) cell wall interference—compounds could inhibit the synthesis of structural components like β-glucan and chitin, leading to weakened cell wall integrity; and (iv) hyphal growth interference—particularly relevant for *A. niger*, where certain polar constituents may affect hyphal extension and spore formation. Critically, it must be emphasized that the disc diffusion assay employed in this study does not distinguish between fungicidal, fungistatic, or target-specific effects; it only measures the net zone of growth inhibition. Therefore, while the concentration-dependent responses and extract-specific activity patterns are consistent with these proposed pathways, they do not constitute direct evidence for any specific molecular mechanism. The differential susceptibility—*C. albicans* showing greater susceptibility to SF-EAE and SF-ME, while SF-AqE performed relatively better against *A. niger*—tentatively suggests that different compound classes (e.g., flavonoid aglycones vs. polar glycosides) may have distinct antifungal preferences; however, this interpretation requires confirmation through bioassay-guided fractionation and mechanistic studies (e.g., ergosterol quantification, membrane integrity assays, and fungal enzyme inhibition tests). Furthermore, as noted in the antibacterial section, formal statistical correlation analyses between TPC/TFC values and antifungal zone diameters were not performed. The observed parallel trends between phytochemical content and antifungal activity are therefore descriptive observations, not statistically validated correlations. The inference that alkaloids are inherently less effective against fungi than phenolics is a speculative one based solely on the lower activity of the crude hexane extract; it does not preclude the possibility that specific purified alkaloids may exhibit potent antifungal activity through

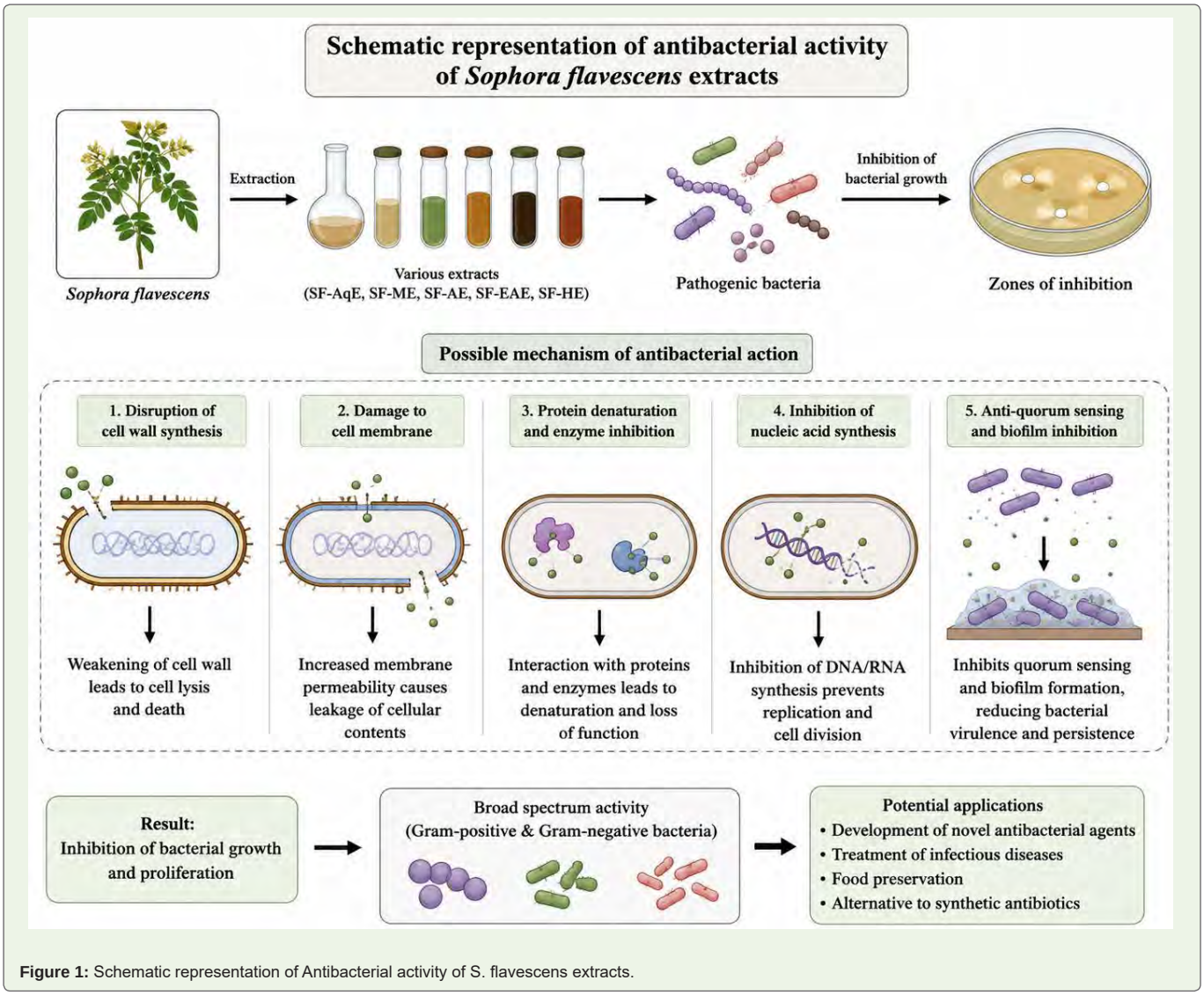

Figure 1: Schematic representation of Antibacterial activity of S. flavescens extracts.

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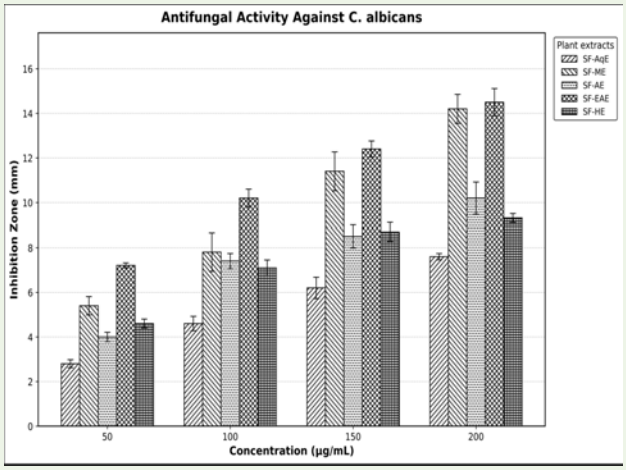

Figure 2: Antifungal activity of S. *flavescens* extracts against *C. albicans*.

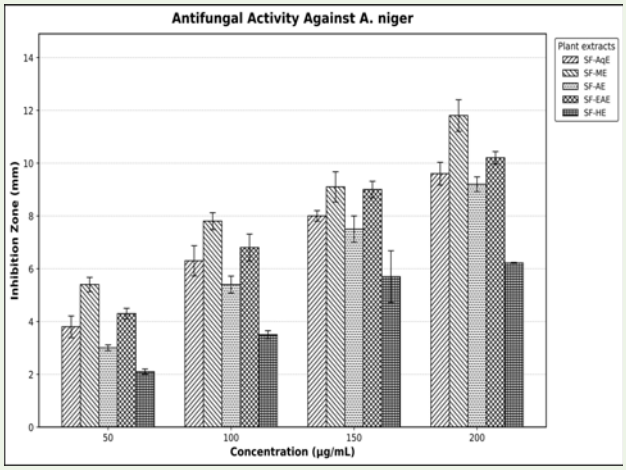

Figure 3: Antifungal activity of S. *flavescens* extracts against *C. albicans*.

distinct mechanisms [18,19,45,46,50].

DPPH radical scavenging activity of *S. flavescens* extracts

The DPPH radical-scavenging activity of all five *S. flavescens* extracts exhibited a clear and progressive concentration-dependent increase over the tested concentration range of 20-100 µg/mL, reflecting that the antioxidant potential of each extract increased with increasing concentration (Figure 5). The scavenging activity of the extracts ranged from 15.34–24.57% at 20 µg/mL and increased substantially to 60.24–83.19% at 100 µg/mL. This concentration-dependent response shows that all extracts possess the ability to neutralize DPPH radicals under the assay conditions, although their antioxidant effectiveness varied according to the extraction solvent. Among the five extracts, SF-ME demonstrated the highest DPPH radical-scavenging activity at all tested concentrations, increasing from 24.57% at 20 µg/mL to 41.28%, 58.47%, 71.84%, and 83.19% at 40, 60, 80, and 100 µg/mL, respectively. The IC_{50} value of SF-ME was 50.15 µg/mL, which was the lowest among all extracts, demonstrating the highest radical-scavenging potency in this particular assay. The strong activity of SF-ME may be attributed to the efficient extraction of a broad spectrum of antioxidant constituents by methanol, including phenolic compounds, flavonoids, flavonoid glycosides, and other polar secondary metabolites. The SF-EAE extract exhibited the second-highest antioxidant activity, with DPPH scavenging increasing from 21.39% at 20 µg/mL to 71.08% at 100 µg/mL. Its IC_{50} value was 62.80 µg/mL, reflecting greater antioxidant potency in this assay than the remaining extracts except SF-ME. The relatively high activity of SF-EAE may be associated with the extraction of moderately polar phenolic and flavonoid constituents by ethyl acetate. Although its activity remained lower than that of SF-ME, the strong radical-scavenging capacity of SF-EAE suggests that several ethyl acetate-soluble constituents may contribute substantially to the antioxidant potential of *S. flavescens*. The difference between SF-ME and SF-EAE also suggests that the observed DPPH scavenging activity may depend not only on the total concentration of phenolic and flavonoid compounds but also on their chemical composition, structural characteristics, and possible synergistic interactions. The SF-AqE extract showed moderate DPPH radical-scavenging activity, increasing progressively from 16.78% at 20 µg/mL to 65.35% at 100 µg/mL. The IC_{50} value of SF-AqE was 69.46 µg/mL, placing it third in terms of antioxidant potency in this system. The observed activity may be attributed to water-soluble phenolic compounds, flavonoid glycosides, and other polar antioxidant constituents extracted into the aqueous fraction. Although SF-AqE was less effective than SF-ME and SF-EAE, its substantial increase in activity with increasing concentration indicates that the aqueous fraction contains constituents that exhibit efficient DPPH-scavenging capacity at higher concentrations. The SF-AE extract exhibited a comparable but slightly lower antioxidant response, with scavenging activity increasing from 18.54% at 20 µg/mL to 62.53% at 100 µg/mL. Its IC_{50} was calculated as 73 µg/mL. The activity of SF-AE was higher than that of SF-AqE at the lowest concentration but remained lower at the higher concentrations, resulting in a slightly higher IC_{50}. The SF-HE extract exhibited the lowest antioxidant potency, although measurable DPPH radical-scavenging activity was observed throughout the tested concentration range. Its activity increased from 15.34% at 20 µg/mL to 28.91%, 37.57%, 54.35%, and 60.24% at 40, 60, 80, and 100 µg/mL, respectively. The IC_{50} value of SF-HE was 74.82 µg/mL, representing the highest IC_{50} among the five extracts. Interestingly, SF-HE showed a pronounced increase in scavenging activity between 60 µg/mL (37.57%) and 80 µg/mL (54.35%). This marked increase may indicate that certain lipophilic

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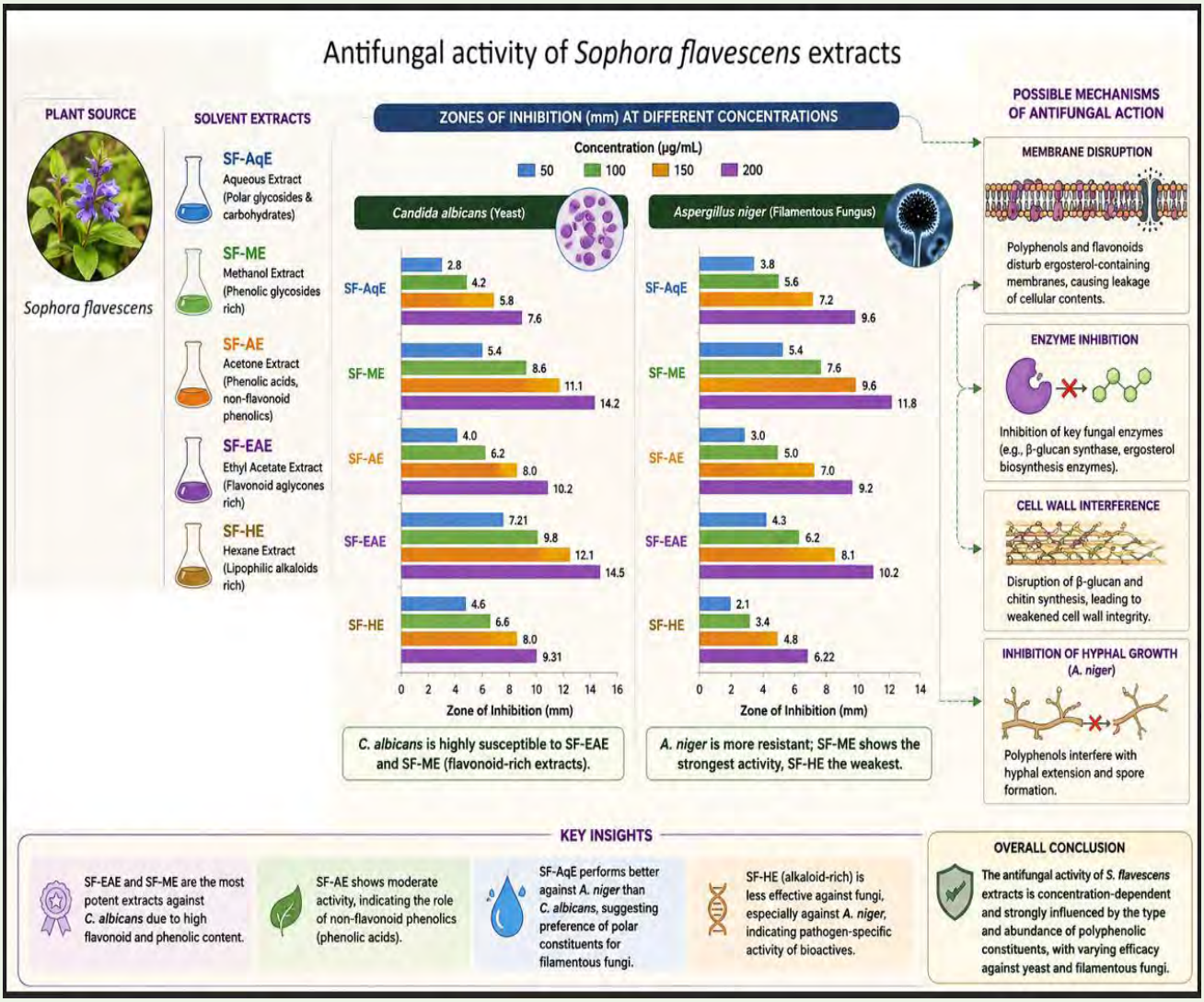

Figure 4: Schematic representation of antifungal activity of S. *flavescens* extracts.

constituents become more effective in radical neutralization at higher concentrations —though this remains speculative without fractionation studies. The measurable DPPH activity of the hexane extract further raises the possibility that antioxidant activity is not exclusively associated with conventional polar phenolic compounds and may also involve non-polar secondary metabolites capable of hydrogen donation, electron transfer, radical stabilization, or other antioxidant mechanisms.

The IC_{50} values obtained by two-point interpolation between the concentrations immediately below and above 50% inhibition provide a quantitative comparison of the antioxidant potency of the extracts. The lowest IC_{50} was observed for SF-ME (50.15 µg/mL), followed by SF-EAE (62.80 µg/mL), SF-AqE (69.46 µg/mL), SF-AE (73.00 µg/mL), and SF-HE (74.82 µg/mL). Therefore, the overall antioxidant potency based on IC_{50} followed the order SF-ME > SF-EAE > SF-AqE > SF-AE > SF-HE. A lower IC_{50} represents a greater ability of the extract to scavenge DPPH radicals under the assay conditions; accordingly, SF-ME was the most potent among the fractions tested in this system.

It is important to emphasize that while the ranking of DPPH scavenging potency broadly paralleled the TPC/TFC order (ME > EAE > AqE > AE > HE), formal statistical correlation analyses between TPC/TFC values and IC_{50} or % scavenging were not performed. Therefore, the observed parallels are descriptive trends, not statistically validated correlations. The inferences that phenolic/flavonoid constituents are primarily responsible for the activity, and that specific mechanisms (e.g., hydrogen atom transfer vs. electron transfer) are involved, remain hypotheses requiring further bioassay-guided fractionation, compound isolation, and mechanistic kinetic

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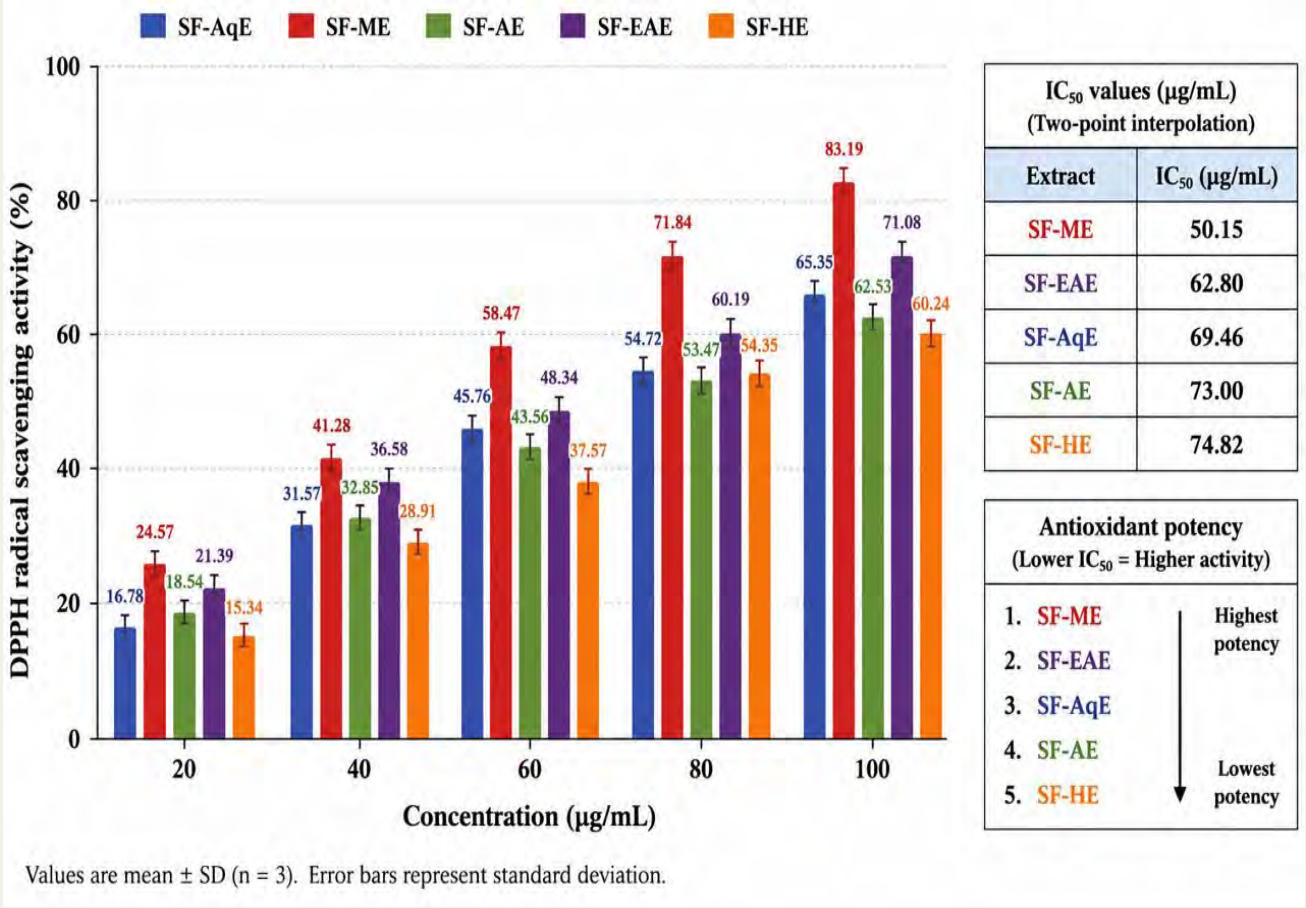

Figure 5: DPPH radical-scavenging activity of different S. *flavescens* extracts.

studies for confirmation [20,21,22,47,48,49,50].

ABTS radical scavenging activity of *S. flavescens* extracts

The ABTS•$^{+}$ radical scavenging activity of all five extracts exhibited a strong and progressive concentration-dependent increase across the 20–100 µg/mL range, with values spanning from 14.23% to 72.35%, demonstrating the robust antioxidant capacity of *S. flavescens* under the assay conditions (Figure 6). Notably, the activity profile differed markedly from the DPPH assay, revealing distinct activity patterns among the solvent fractions. At the highest concentration tested (100 µg/mL), SF-EAE (ethyl acetate) emerged as the most potent scavenger with 72.35% inhibition, closely followed by SF-HE (hexane) at 70.13% and SF-ME (methanol) at 69.85%, with only a narrow margin separating the top three performers. The superior activity of SF-EAE was consistent with its highest total phenolic content (17.4 mg GAE/g) and flavonoid content (11.2 mg QE/g), which may suggest that the phenolic aglycones and flavonoids preferentially extracted by ethyl acetate are efficient at reducing the ABTS cation radical—potentially through electron transfer mechanisms.

The most striking observation, however, is the remarkable activity of SF-HE (70.13%), which ranked last in the DPPH assay (60.24%) but jumped to second position in ABTS scavenging—despite containing zero phenolics or flavonoids. This dramatic reversal raises the interesting possibility that the lipophilic alkaloids uniquely present in the hexane fraction may possess notable capacity to scavenge the ABTS radical cation, potentially through electron transfer (ET) mechanisms that may be favourable for certain nitrogen-containing alkaloid structures. In contrast, DPPH scavenging is generally understood to rely more heavily on hydrogen atom transfer (HAT) facilitated by phenolic hydroxyl groups—a mechanistic distinction that may explain why phenolic-rich extracts excelled in that assay. This mechanistic interpretation, however, is based on established literature and observed activity patterns; direct evidence for HAT versus ET pathways in these specific crude extracts remains circumstantial at this stage and warrants further investigation using purified compounds and mechanistic kinetic studies. This observed reversal underscores

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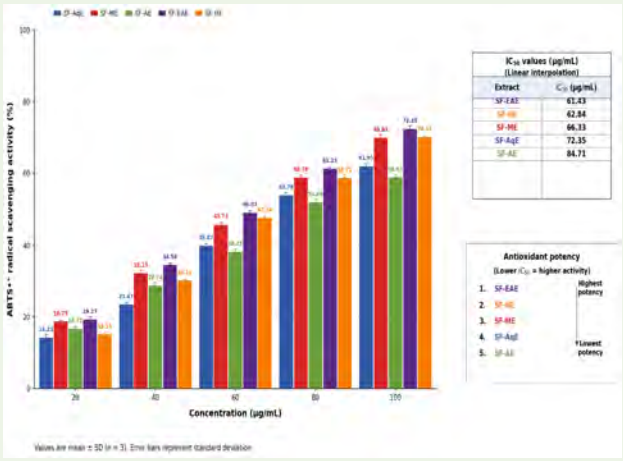

Figure 6: ABTS radical scavenging activity of S. flavescens extracts.

the importance of employing multiple radical-generating systems to comprehensively evaluate antioxidant potential.

SF-ME (69.85%) performed robustly and consistently, reflecting its broad-spectrum extraction of both polar phenolic glycosides and moderately polar aglycones, which collectively may address both ET and HAT-type radical neutralization. The aqueous extract SF-AqE (61.95%) and acetone extract SF-AE (58.83%) demonstrated moderate activities, which were broadly consistent with their lower phenolic and flavonoid loads, with SF-AE's lack of flavonoids (TFC = 0) possibly contributing to its comparatively lower performance. Notably, the ABTS activity ranking at 100 µg/mL (EAE > HE > ME > AqE > AE) contrasts sharply with the DPPH ranking (ME > EAE > AqE > AE > HE). This reversal, particularly the rise of SF-HE, highlights that evaluating antioxidant potential using a single assay is insufficient; the chemical nature of the radical and the extraction solvent critically determine the observed bioactivity profile. Furthermore, the gap between SF-EAE (72.35%) and SF-ME (69.85%) in ABTS is narrower than in DPPH, tentatively suggesting that while methanol may extract a more hydrogen-donating pool of phenolic glycosides, ethyl acetate may extract a more electron-donating pool of aglycones and simpler phenolics. However, this remains a speculative inference based solely on the observed activity differences, not on direct mechanistic evidence. The substantial activity of SF-HE against the ABTS radical further suggests that the alkaloid-enriched fraction may contribute to the overall antioxidant capacity of the plant under these assay conditions; however, the specific compounds responsible and their precise mechanisms remain to be identified through bioassay-guided fractionation.

The IC_{50} values further supported the observed differences in ABTS radical scavenging potency among the *S. flavescens* extracts. Based on two-point linear interpolation between the 40 and 60 µg/mL concentrations, SF-EAE exhibited the lowest IC_{50} value (61.43 µg/mL), indicating the highest apparent ABTS scavenging potency among the five extracts. SF-HE showed a closely comparable IC_{50} of 62.84 µg/mL, followed by SF-ME (66.33 µg/mL), SF-AqE (72.35 µg/mL), and SF-AE (84.71 µg/mL). Thus, the IC_{50}-based potency order was SF-EAE > SF-HE > SF-ME > SF-AqE > SF-AE, which was broadly consistent with the activity observed at the higher concentrations, particularly the strong performance of SF-EAE and the unexpectedly high activity of SF-HE. The relatively low IC_{50} of SF-HE, despite its absence of detectable phenolics and flavonoids, further emphasizes that its ABTS-scavenging activity may involve other constituents, potentially including alkaloids.

It is critically important to emphasize that while the ABTS activity ranking broadly paralleled the TPC/TFC order for some extracts (notably SF-EAE and SF-ME), formal statistical correlation analyses between TPC/TFC values and % scavenging or IC_{50} were not performed. The observed parallels are therefore descriptive trends, not statistically validated correlations. Furthermore, the proposed mechanistic distinctions between HAT (for DPPH) and ET (for ABTS) are inferences drawn from the differential activity patterns and established phytochemical literature; they do not constitute direct experimental proof. Future studies employing purified compounds, kinetic analyses, and structure-activity relationship investigations are essential to confirm these proposed mechanisms and to definitively attribute the observed activities to specific phytochemical classes [20,21,22,47,48,49,50].

Hyaluronidase Inhibitory Activity

The hyaluronidase inhibition activity of the five *S. flavescens* extracts demonstrated a consistent concentration-dependent escalation across the 50–250 µg/mL range, with inhibition percentages spanning from 15.31% to 52.42% (Figure 7). This dose-

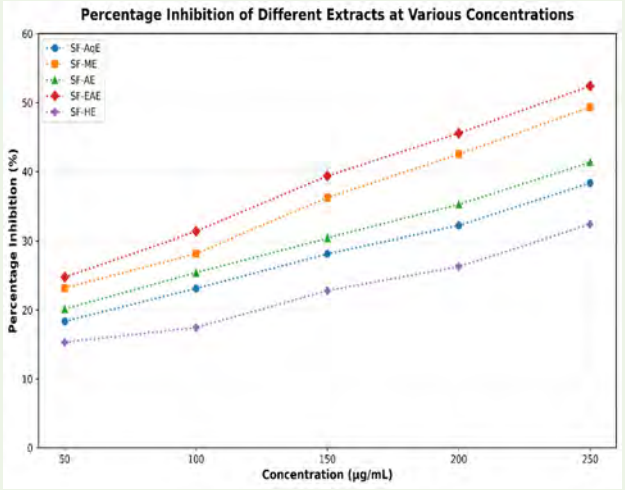

Figure 7: Hyaluronidase inhibitory activity of S. flavescens extracts.

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responsive behavior shows that the anti-hyaluronidase potential of this plant increases with concentration under the assay conditions. At the highest tested concentration (250 µg/mL), SF-EAE exhibited the most potent inhibition at 52.42%, closely followed by SF-ME at 49.35%, SF-AE at 41.34%, SF-AqE at 38.34%, and SF-HE at 32.34%. This ranking broadly paralleled the total phenolic and flavonoid content profiles previously established. This parallel trend is suggestive, suggesting that polyphenolic constituents may play a role in interfering with the enzymatic breakdown of hyaluronic acid. However, formal statistical correlation analyses between TPC/TFC values and % inhibition were not performed; therefore, these comparisons represent descriptive observations rather than statistically validated correlations.

The activity of SF-AE (41.34%) and SF-AqE (38.34%) was moderate and comparable, reflecting their distinct phytochemical compositions. SF-AE, despite containing measurable phenolics, completely lacks flavonoids; this observation raises the possibility that non-flavonoid phenolics such as phenolic acids and simple coumarins may contribute to hyaluronidase inhibition, albeit with potentially lower efficacy than flavonoids. SF-AqE, on the other hand, captures polar phenolic glycosides and carbohydrates but misses the aglycone fraction, which may contribute to its reduced overall inhibitory effect.

Notably, the hexane extract SF-HE retained considerable activity (32.34%) despite containing zero phenolics or flavonoids. This finding suggests that lipophilic alkaloids may also contribute to hyaluronidase inhibition. Alkaloids, with their nitrogen-containing heterocyclic structures, are hypothesized to interact with the enzyme through electrostatic interactions and hydrogen bonding with acidic or polar residues, potentially offering a complementary non-phenolic inhibition pathway. Nevertheless, these proposed structure-activity relationships are speculative at this stage, and direct enzyme kinetic studies with isolated compounds are required to validate the underlying mechanisms [34,35,50,51].

Conclusions

The present study successfully demonstrated that *Sophora flavescens* is a rich source of diverse bioactive phytochemicals with significant antibacterial, antifungal, antioxidant, and hyaluronidase inhibitory activities. Through systematic solvent extraction using hexane, ethyl acetate, acetone, methanol, and aqueous solvents, we established a clear polarity-dependent extraction profile, with SF-EAE (ethyl acetate) emerging as the most potent extract for phenolic and flavonoid recovery, antibacterial activity against *Bacillus subtilis*, and hyaluronidase inhibition. SF-ME (methanol) proved to be the most exhaustive extract, yielding the highest DPPH radical scavenging activity and robust broad-spectrum antimicrobial effects. Notably, SF-HE (hexane) exhibited remarkable activity against Gram-negative bacteria (*E. coli*, *P. aeruginosa*) and *S. aureus* despite containing no phenolics or flavonoids, confirming that alkaloids are key contributors to antibacterial efficacy against these pathogens. The mechanistic studies revealed that the extracts exert their effects through multiple interconnected pathways, including membrane disruption, cell wall synthesis inhibition, enzyme inhibition, nucleic acid interference, anti-quorum sensing, and biofilm inhibition. The antioxidant assays demonstrated that while phenolic compounds primarily contribute to hydrogen atom transfer (DPPH), alkaloids show significant electron transfer capacity (ABTS), highlighting the complementary nature of these phytochemical classes. The hyaluronidase inhibition results further established the potential of *S. flavescens* extracts in preserving extracellular matrix integrity, offering promising applications in skin health, anti-aging formulations, and joint health management. The differential activity patterns observed across the five solvent extracts underscore the critical importance of solvent selection in optimizing bioactive compound recovery and achieving targeted therapeutic outcomes. This comprehensive investigation provides a strong scientific rationale for the traditional use of *S. flavescens* in TCM and establishes a robust foundation for its development as a multitarget therapeutic agent.

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Conflicts of Interest

All authors declare that there are no personal and financial conflicts of interests to declare. All authors read and approved the manuscript to publish.

Ethics and Consent to Participate declarations

No animals/humans used for the present study.

Data availability Statement

No datasets were generated or analysed during the current study.

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