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Chemical Composition and Antibacterial Activity of Senna Alexandrina Mill methanol extracts: In Vitro and In Silico Studies

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Abstract

Historical reliance on Tinnevelly senna (Senna alexandrina Mill.) within traditional pharmacopoeias underscores its potential as a reservoir for novel antimicrobial scaffolds. This investigation dissects the chemical architecture and bactericidal efficacy of methanolic leaf and pod extracts through a multi-analytical lens. High-Performance Liquid Chromatography (HPLC) revealed that sennosides A and B constitute the primary bioactive markers, with leaf matrices exhibiting superior concentrations—specifically 25.3 mg/g and 27.8 mg/g, respectively. Biological assays demonstrated significant inhibitory zones against Escherichia coli and Pseudomonas aeruginosa. Computational modeling reinforced these findings; sennoside B displayed a notable binding affinity of -8.7 kcal/mol against the E. coli DNA gyrase B subunit, suggesting a targeted mechanical disruption of bacterial replication. These results advocate for the strategic integration of senna-derived glycones into the development of sustainable antibacterial therapies.

Keywords: Senna alexandrina, antibacterial activity, Molecular docking, Sennoside

* Introduction

The bedrock of global therapeutic practices remains firmly

rooted in botanical medicine, with nearly 85% of the human population[1-3] —particularly within Asian and African demographics—relying on traditional herbal systems for primary healthcare.[4, 5] This pervasive dependence is rarely a matter of mere preference; rather, it is a pragmatic response to the prohibitive costs and logistical scarcity of modern synthetic pharmacology.[6, 7]As the specter of multidrug-resistant pathogens looms over contemporary medicine.

the World Health Organization (WHO) has increasingly looked toward the vast chemical diversity of the plant kingdom as a primary frontier for discovering novel antimicrobial scaffolds.[8]

Within the Fabaceae lineage, Senna alexandrina Mill commonly referred to as Indian or Tinnevelly senna—has long been recognized as one of the most prominent and scientifically significant botanical species. [9] Cultivated across the global tropics, its presence is ubiquitous in the traditional canons of Ayurveda, Unani, and Siddha medicine.[10] While its historical reputation is largely anchored in its efficacy as a purgative, the plant's pharmacological profile is significantly more complex. The medicinal "engine" of S. alexandrina resides in its sennosides: a specific class of anthraquinone glycosides synthesized within the leaves and pods. [11] Beyond their well-known laxative properties, these compounds exhibit a multifaceted bioactivity spectrum, ranging from anti-inflammatory and antioxidant effects to potent anticancer and antimicrobial signatures. [12] This bioactivity is supported by a dense matrix of secondary metabolites, including alkaloids, flavonoids, polyphenols, and terpenoids, which historically have been deployed to combat conditions as diverse as jaundice, dysentery, and viral infections. [13,14]

Despite this storied history of use, the specific antibacterial mechanisms of Senna populations—particularly those indigenous to the Al-Jabal Al-Akhdar region of northeastern Libya—remain insufficiently validated by rigorous empirical data. The present study addresses this knowledge gap by employing a dual-methodological approach. By utilizing a methanolic maceration technique, we extracted bioactive fractions from both the foliage and the fruit of the plant. These extracts were subjected to quantitative HPLC analysis to isolate sennosides A and B, followed by in vitro well diffusion assays to determine their bactericidal

threshold. To bridge the gap between experimental observation and molecular mechanism, we further employed in silico docking simulations. By modeling the interactions between these sennosides and the DNA gyrase B subunits of Staphylococcus aureus (PDB: 6TTG) and Escherichia coli (PDB: 6F86), this research seeks to elucidate how these botanical compounds disrupt bacterial replication, thereby offering a structural blueprint for future pharmaceutical development.

*** Experimental Methodology**

*** Botanical Procurement and Authentication**

Biological samples of Senna (foliage and fruit pods) were harvested during the peak seasonal window of June 2024 from the Al-Jabal Al-Akhdar topography in northeastern Libya. To ensure taxonomic rigor, Dr. Babiker of the Department of Pharmacognosy at Omar Al-Muktar University performed the formal authentication of voucher specimens. Initial processing involved desiccation under controlled shade conditions until the biomass reached a stable, constant mass. Once dried, the distinct anatomical parts were mechanically pulverized and sequestered in a desiccator to prevent hygroscopic degradation prior to extraction.

*** Reagents and Analytical Standards**

Primary reference standards of sennosides A and B with a stated purity of 98% were procured from Sigma-Aldrich (USA). HPLC-grade methanol (99.9%) was supplied by Patel Chemicals, India, whereas ultrapure water was prepared using a Milli-Q purification unit manufactured by Millipore, USA. Additional analytical-grade solvents employed throughout the study were obtained from National Analytical Corporation, India.

For the biological assays, a panel of indicator pathogens was utilized, encompassing Gram-positive strains (Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 1070) and Gram-negative counterparts (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853). These isolates were maintained by the Laboratory of Biomedical Science at Omar Al-Mukhtar University.

*** Extraction Protocols and Sample Refinement**

Solid-liquid extraction was initiated by immersing 50 g of the pulverized plant material in 500 mL of absolute methanol. This maceration proceeded for 24 hours at

ambient temperature. Following extraction, the solvent was stripped using rotary evaporation, and the resulting residue was air-dried to yield a stable biomass.

For HPLC characterization, 10 mg of the crude extract was reconstituted in 1 mL of methanol and passed through a 0.45 µm PVDF membrane. While immediate quantification followed, reserve aliquots were archived at -4°C to preserve phytochemical integrity

*** High-Performance Liquid Chromatography (HPLC) Quantification**

Analysis of the filtered samples was conducted using an HPLC platform (Agilent 1260 series, Agilent Technologies, Santa Clara, CA, USA), into which 20 µL of each extract solution (10 mg/mL) and reference standard solution containing sennoside A and sennoside B (1 mg/mL) were introduced. Chromatographic separation was achieved on a C18 analytical column (4.6 × 250 mm, 5 µm particle size). The elution process employed an isocratic mobile phase composed of HPLC-grade methanol and water acidified with 0.1% formic acid in an 80:20 (v/v) ratio, while the system was operated at a constant flow rate of 1 mL/min. was elucidated using an Agilent 1260 series HPLC architecture. We employed an isocratic elution strategy on a C18 stationary phase (4.6 × 250 mm, 5 µm). The mobile phase consisted of a methanol-to-acidified water (0.1% formic acid) ratio of 80:20 (v/v), maintained at a persistent flow rate of 1 mL/min.[15] Analytes were monitored at a wavelength of 230 nm. By injecting 20 µL of both the 10 mg/mL extracts and the 1 mg/mL standards, we determined the sennoside the sennoside content of the senna extracts was quantified using the following formula:

$$\text{Concentration of the sample (g/ml)} = \frac{\text{Peak area the sample}}{\text{Peak area of the standard}} \text{x1000}$$

*** Assessment of Antibacterial activity**

The growth-inhibitory potential of the extracts was evaluated via the agar well diffusion technique.[16] Dried residues were solubilized in 10% dimethylsulfoxide (DMSO) to a standardized concentration of 20 mg/mL. Mueller-Hinton agar plates were prepared via autoclaving at 121°C for 15 min and subsequently inoculated for 30 min with the target bacterial suspensions.

Uniform 10 mm diameter wells were excised from the agar and charged with 200 µL of the extract. To facilitate pre-incubation diffusion,

plates remained at room temperature for 30 minutes before an 18-hour incubation period at 37°C.

Ciprofloxacin (10 µg/mL) and 10% DMSO served as the positive and negative benchmarks, respectively. Antibacterial potency was quantified by measuring the diameter of the clear zones of inhibition (ZOI) in millimeters. All experiments were conducted in triplicate.

*** Computational Molecular Docking**

To investigate the molecular mechanisms underpinning the observed antibacterial activity, we performed in silico docking simulations. We focused on the binding affinities of Sennosides A, B and the reference drug (ciprofloxacin) against two critical enzymatic targets: S. aureus DNA gyrase B (PDB ID: 6TTG) and E. coli DNA gyrase B (PDB ID: 6F86). These targets were selected due to their essential role in bacterial replication and their status as established sites for clinical antibiotics.

Molecular architectures were drafted using ChemDraw Professional 2023, while protein coordinates were retrieved from the RSCB Protein Data Bank (https://www.rcsb.org/) in the PDB format (Table 1). Target preparation was executed within the Discovery Studio (v2025) environment as outlined by Mashraqi, et al. (2023).[17]. The actual docking maneuvers were performed using AutoDock Vina 4.2.6. We prioritized conformers exhibiting the lowest binding free energy, visualizing the specific ligand-amino acid interactions through Discovery Studio.

*** Statistical Refinement**

All experimental iterations were performed in triplicate. Data are articulated as the mean \pm standard deviation. Statistical significance across the inhibition percentages was scrutinized via one-way Analysis of Variance (ANOVA), where a p-value of less than 0.05 was established as the threshold for statistical relevance.

*** Results and Discussion**

*** Efficiency of Phytochemical Recovery**

The extractable yield of botanical samples is fundamentally dictated by the interplay between the plant matrix and the solvent's thermodynamic properties. In this investigation, methanolic extraction of Senna biomass revealed subtle variations in mass recovery. The leaf tissue yielded 23.90 mg/100 g, marginally surpassing the pod yield of 20.15 mg/100 g (Table 2). These discrepancies highlight the influence

of anatomical tissue density and the localized concentration of secondary metabolites on the overall extraction efficiency.

*** Chromatographic Profiling of Anthraquinone Glycosides**

Utilizing a high-sensitivity HPLC framework, we achieved rapid resolution of the primary bioactive constituents, Sennosides A and B, in under 10 minutes. The elution sequence was characterized by high peak symmetry and reproducible retention times, with Sennoside B emerging at 4.71 minutes and Sennoside A at 6.49 minutes. Figure 1 shows the chromatograms obtained from the methanol extracts and reference compounds. The peaks corresponding to the individual compounds were symmetrical and well-resolved from those of the other co-extracted compounds. This method ensures good column performance and reproducible retention times. The quantification process used an external standard method. Quantitative assessment demonstrated that the leaf-derived extract possessed greater concentrations of both sennoside A and sennoside B than the pod extract. Specifically, the levels detected in the leaf extract reached 25.3 mg/g and 27.8 mg/g for sennosides A and B, respectively, whereas the pod extract exhibited corresponding values of 22.2 mg/g and 26.7 mg/g (Table 2). Our data aligns with previous literature on Indian Senna variants, which reported similar distributions of these anthraquinones, thereby validating the phytochemical profile of the Libyan Al-Jabal Al-Akhdar specimens.[15]

*** Evaluation of bactericidal potency**

Addressing the global threat of foodborne pathogens, we assessed the extracts against a spectrum of microbial targets. The methanolic fractions Table 3 exhibited robust inhibitory activity, generating zones of inhibition (ZOI) between 14.3 mm and 22.2 mm. Escherichia coli proved exceptionally vulnerable, showing the highest sensitivity to the leaf extract (22.2 mm). Furthermore, the leaf extract demonstrated significant efficacy against Pseudomonas aeruginosa (21.1 mm) and Staphylococcus aureus (16.1 mm), while both plant parts showed uniform activity against Bacillus subtilis (15 mm).

Previous studies have reported the antibacterial properties of senna leaf extract using various solvents, such as acetone, dichloromethane, and petroleum ether. The acetone extract showed activity against all laboratory isolates, with a 6 mm

inhibition zone for S. aureus and a 10 mm zone for E. coli. At a concentration of 5 mg/mL, the dichloromethane extract demonstrated antibacterial activity against S. aureus and E. coli, producing inhibition zones of 8 mm and 10 mm, respectively.15 Comparable findings were reported for Cassia angustifolia, where the aqueous extract prepared from leaves at 10 mg/mL generated the widest inhibition zone against B. subtilis (15.33 mm). Moreover, the petroleum ether leaf extract of C. angustifolia at the same concentration exhibited superior antibacterial performance against S. aureus, with an inhibition diameter of 15.66 mm, whereas the aqueous extract produced a smaller zone measuring 12 mm.21. Relative to earlier investigations, the present study demonstrated that methanolic senna extracts at 10 mg/mL produced the most pronounced antibacterial effects. The enhanced activity may be associated with the polarity characteristics of the extraction solvent, which likely facilitated greater recovery of sennosides and consequently improved antimicrobial potency. Previous research has consistently highlighted the critical influence of solvent polarity on both extraction yield and the biological properties of phytochemical constituents. In this regard, Avetis et al. (2025) reported a strong association between solvent polarity, extraction efficiency, and the biological activity of compounds isolated from plant matrices.22 These observations emphasize that selecting a solvent with polarity compatible with the chemical profile of target metabolites is essential for maximizing extraction performance and optimizing the recovery of pharmacologically active compounds. Our results notably exceed the inhibitory zones reported in studies using less polar solvents like petroleum ether or dichloromethane. This enhanced efficacy is likely a function of methanol's superior ability to solubilize high concentrations of sennosides. The strong correlation between solvent polarity and biological activity, as discussed in recent scholarship, underscores the necessity of optimizing extraction parameters to maximize the recovery of pharmacologically active molecules.

*** Computational Insights into Molecular Interaction**

To bridge the gap between in vitro results and molecular mechanisms, docking simulations targeted the bacterial DNA gyrase

B—an enzyme central to DNA replication via ATP hydrolysis. [23]

Docking studies were conducted to evaluate the antibacterial activity of sennoside A and B from senna extract, which were identified as major constituents by HPLC. Binding affinities are presented in Table 4

Intriguingly, Sennoside B exhibited superior binding affinities (-8.3 to -8.7 kcal/mol) compared to the clinical benchmark, ciprofloxacin (-7.3 to -7.7 kcal/mol). Sennoside A also displayed competitive binding scores (-7.5 to -8.0 kcal/mol). Molecular docking investigations demonstrated favorable binding affinities between the studied compounds and the target receptors. This interaction pattern may be explained by the presence of polar amino acid residues within the receptor active sites, which facilitated multiple intermolecular interactions with the ligands, including hydrogen bonding, van der Waals interactions, pi-anion associations, and carbon–hydrogen bonding within the gyrase enzymes of S. aureus and E. coli (Figures 2 and 3). The relatively high abundance of these constituents in the extract may also have contributed to the observed activity. Nevertheless, evaluation of the docking scores suggested that sennoside A exhibited stronger binding performance and greater affinity toward the target proteins than sennoside B.

Similar findings were reported by,[15] who studied the contribution of sennoside A and B to the antibacterial activity of S. alexandrina. Additionally, Agi et al. (2022) found that sennoside A and sennoside B exhibited strong binding affinities, stable interactions, and potential as antibacterial inhibitors.[24] Belkheit (2025) also reported that sennosides act as inhibitors of bacterial RNA polymerase enzymes and gastric receptor (ATPASE proton pump).[25] The current investigation indicated that sennoside B had a higher binding affinity for DNA gyrase B of S. aureus and E. coli compared to ciprofloxacin. Therefore, this study suggests the isolation or synthesis of sennoside B to develop a biologically active molecule that can act as an antibacterial agent against bacterial infections.

The computational data suggests that the synergistic presence of these compounds in the extract drives the observed antibacterial effect. Specifically, the exceptional binding profile of Sennoside B identifies it as a prime candidate for further drug development. These findings resonate with contemporary research suggesting that sennosides

may function as multi-target inhibitors, potentially disrupting both DNA gyrase and RNA polymerase activities.

*** Statistical Inference**

A one-way ANOVA confirmed that the observed variations in bacterial inhibition across different extracts were statistically significant ($p < 0.05$). This rigorous analysis reaffirms that the methanolic extracts of Senna possess potent, quantifiable antibacterial properties rooted in their specific phytochemical makeup.

*** Conclusion**

We conducted a study synthesized phytochemical profiling with in vitro and in silico evaluations to elucidate the antibacterial potential of Senna leaf and fruit pod extracts. Chromatographic quantification established that sennosides constitute the primary bioactive matrix in both anatomical parts, though a distinct quantitative superiority was observed in the foliage. Specifically, the leaf extract sequestered significant concentrations of Sennoside B (27.8 mg/g) and Sennoside A (25.3 mg/g).

Biological assays against a panel of clinically relevant pathogens (S. aureus, B. subtilis, E. coli, and P. aeruginosa) demonstrated robust growth inhibition across all strains. The leaf extract's heightened potency, particularly against Gram-negative isolates, correlates directly with its enriched anthraquinone glycoside profile. These empirical findings were further reinforced by molecular docking simulations, which revealed high-affinity binding interactions between sennosides and the microbial Gyrase B subunit.

Ultimately, this research positions sennosides A and B not merely as conventional laxatives, but as credible scaffolds for the development of natural antibacterial agents. The transition from crude botanical extracts to refined therapeutic applications offers a promising trajectory for the pharmaceutical and food preservation industries in their ongoing struggle against microbial resistance.

*** Conflict of Interest**

The authors formally declare that no competing financial interests or personal relationships influenced the execution or reporting of this research.

*** Author's Declaration**

The authors affirm the originality of the data presented herein. Any liability or ethical considerations arising from the content of this manuscript are the sole responsibility of the signatories.

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* List of Table

Table 1. Molecular docking target of bacterial target proteins, PDB ID's, and active site coordinates.

Target	PDB ID	Coordinates		
		X	Y	Z
Gyrase B subunit of S. aureus	6TTG	6.995	7.171	3.471
Gyrase B subunit of E. coli	1KZN	20.154	23.677	35.508

Table2. Extraction efficiency and concentrations of sennosides A and B detected in senna-derived extracts.

Parameter	**Leaf**	**Pods**
Extraction yield (g/100 g)	23.90	20.15
Sennoside A (mg/g)	25.3	22.2
Sennoside B (mg/g)	27.8	26.7

Table 3. Antibacterial activity of senna extracts (10 mg/mL) against selected mmicroorganisms

Microorganisms	**Zone of inhibition (mm)**		
	Leaf	**Pods**	**CF**
S. aureus	16.1 ±1.5	14.3 ± 1.3	22.2 ± 3.2
B. subtilis	15.4 ± 1.3	15.5 ± 1.7	23.5 ± 2.2
E. coli	22.2 ± 2.1	20.3 ± 2.1	33.3 ± 3.8
P. aeruginosa	21.1 ± 2.3	18.9 ± 2.1	27.3 ± 3.2

Values are mean ±S.D of three replicates; CF-Ciprofloxacin (10 µg/mL)

Table 4. Docking results of sennosides A and B with the microbial protein Gyrase B subunit of S. aureus and E. coli

Molecules	**Binding affinity kcal/mol**	
	Gyrase B of S. aureus	Gyrase B of E. coli
Ciprofloxacin	-7.7	-7.3
Sennoside A	-8.5	-8.0
Sennoside B	-8.3	-8.7

* List of Figures

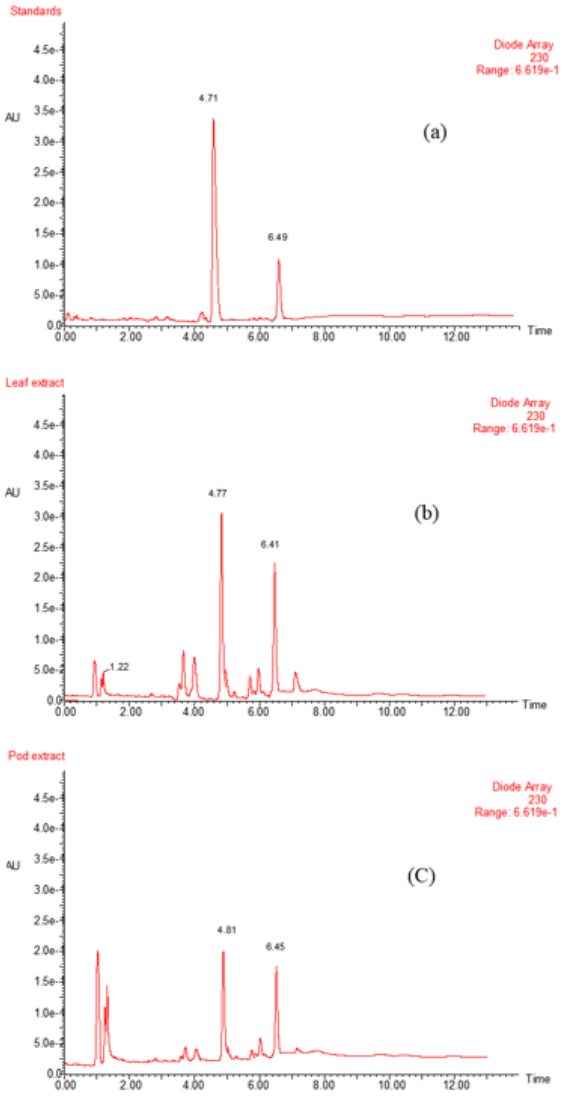

Figure 1. HPLC-based determination of sennosides A and B in methanolic extracts prepared from senna leaves and pods. (a) Reference chromatogram showing sennoside B at Rt 4.71 and sennoside A at Rt 6.49; (b) chromatographic profile of the methanolic leaf extract; (c) chromatographic profile of the methanolic pod extract. .

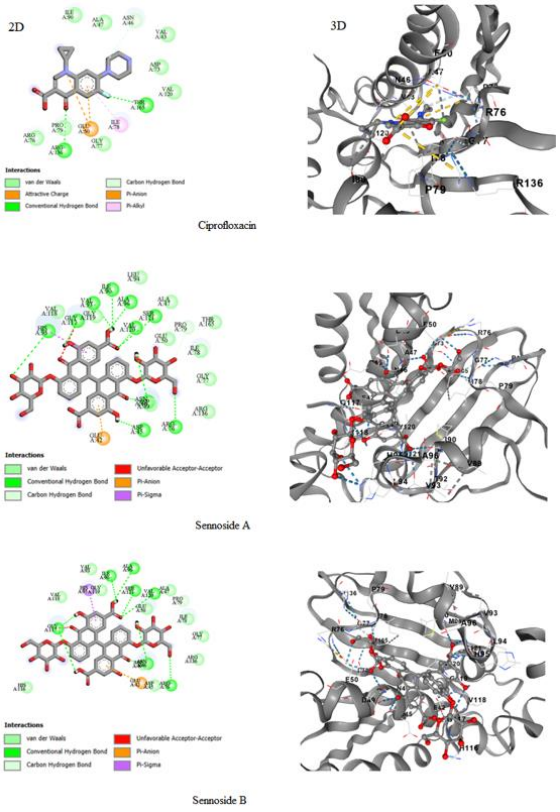

Figure 2. 2D and 3D visualizations of potential interactions between ligands and the active site of the S. aureus DNA gyrase receptor (BDB: 6TTG)

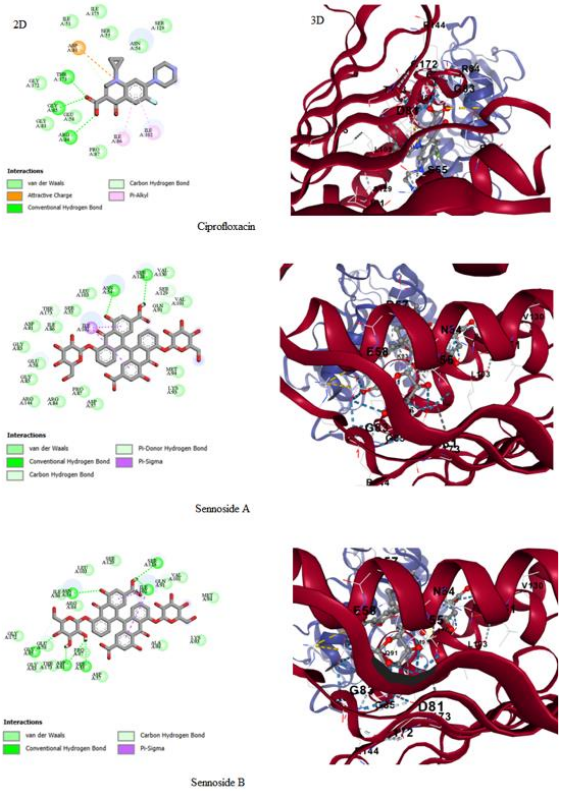

Figure 3. 2D and 3D visualizations of potential interactions between ligands and the active site of the E. coli DNA gyrase receptor (BDB: 1KZN)