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Phytochemical and Antibacterial analysis of *Senna montana* fruits

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A B S T R A C T

This study evaluated the Phytochemical profile and antibacterial potential of *Senna montana* fruits (Pods) extracts against four clinically significant human pathogens. Utilizing a systematic solvent -polarity gradient (Aqueous, Ethanolic, Ethyl acetate and n-Hexane), the investigation revealed diverse array of pharmacologically active therapeutic secondary metabolites within the ethanolic extract yielding the highest concentration of Phenols, tanins, flavonoids. Antibacterial assays against a panel of Human pathogens (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Klebsiella pneumoniae*) demonstrated significant, dose-dependent growth inhibition. The ethanolic extract exhibited superior efficacy, producing Zones of Inhibition (ZOI) of 21.0–23.5 mm at 100 mg/mL. Notably, biostatistical benchmarking via Two-way ANOVA and Tukey's HSD confirmed that the antimicrobial potency of the ethanolic extract is statistically comparable to clinical standards. These findings suggest that *Senna montana* pods serve a profilic reservoir bioaccessible secondary metabolites and as a significant lead for the isolation of bioactive molecules in the pursuit of novel antimicrobial drug discovery.

Introduction

Senna montana (B.Heyne ex Roth) V.Singh (Clade : Mimosoid, Family : Fabaceae) is a large shrub to a small tree, notable by its yellow corymbose flowers and straight, flat pods marked with prominent veins. Basionym is *Cassia montana* B.Heyne ex Roth. Native primarily to Peninsular India and is commonly known as Mountain Cassia or Deccan Senna (Figure 1).

The vernacular names of *Senna montana* in Telugu are Konda thangedu, Paidi thangedu, Pagadi thangedu etc. Generally distributed along the roadside of Madanapalle, Kuppam regions of Chittoor Dist. (Chetty *et al.,*2018; Korlam *et al*. 2019).

Taxonomically, *S. montana* is classified within the Caesalpiniaceae (Leguminosae), a genus characterized by extensive speciation and significant pharmacological utility. The nomenclature of *Senna* has undergone substantial revision since the 18th century; originally, Linnaeus (1753) subsumed the genus under the broader Linnaean *Cassia* concept in *Species Plantarum*, a classification that has since been refined to distinguish the two distinct lineages (Raj *et al.,* 2025). Recent advancements in molecular phylogenetics (Angiosperm

Phylogeny Group, 2016) have led to the taxonomic reclassification of the formerly expansive genus *Cassia* into three monophyletic lineages: *Cassia* (*sensu stricto*), *Senna*, and *Chamaecrista*. Under this revised framework, *Senna montana*, morphologically identified by anthers that dehisce via apical pores and the absence of bracteoles on the pedicel. In contrast, the restricted genus *Cassia* is characterized by staminal filaments that are distinctly curved and medially dilated.

Hemalatha *et al.* (2021) identified *Senna montana* as endemic to specific Southern Peninsular Indian regions—notably the Ardhagiri Hills—their assessment lacked comprehensive population and ecological data. Contrasting literature and field observations suggest a more expansive distribution across the Eastern Ghats and broader South Indian hillocks, indicating that current records may underestimate its true ecological range.

Phytochemical and pharmacological evaluations of *Senna montana* have identified significant bioactive potential across various plant parts. Mittoori *et al.* (2024) reported high concentrations of α-methyl mannofuranoside in the root bark, demonstrating its role as a potent 5-α-reductase and TNF-α-inhibitor. Complementary studies on leaf extracts of *S.montana* have validated their antimicrobial efficacy, specifically against Gram-positive pathogens such as *Bacillus subtilis* and *Staphylococcus aureus* (Hemalatha *et al.,* 2021). Furthermore, solvent-specific screening by Korlam *et al*. (2019) indicated that while carbohydrates remain ubiquitous across various solvent systems, alkaloids are uniquely partitioned into the ethanolic fraction of leaves, stem bark, and root bark of *S. montana*.

These findings underscore the clinical relevance of the genus *Senna*, as recent pharmacological reviews advocate for the integration of these bioactive constituents into modern herbal therapeutics (Raj *et al.,* 2025).

The global escalation of antimicrobial resistance (AMR) has necessitated an urgent transition toward natural, plant-derived alternatives to traditional antibiotics. Recent studies emphasize that traditional medicinal systems, such as Ayurveda and Siddha, offer a wealth of unexplored taxa capable of serving as chemical templates for next-generation drug synthesis (Jones *et al*., 2025; Kumar *et al*., 2025).

Current biopharmaceutical research highlights that secondary metabolites specifically alkaloids, phenolic compounds, and flavonoids exert their antibacterial effects through multi-target mechanisms, including the disruption of bacterial cell walls, inhibition of biofilm formation, and interference with quorum sensing (Arbab *et al*., 2024; Ogofure *et al*., 2025). This multi-pronged action, as noted by researchers, significantly reduces the likelihood of pathogens developing resistance compared to single-target synthetic agents (Mihociu *et al*., 2024; Khare *et al*., 2021).

Fig. 1 A & B. Habitat and Fruits (Pods) of *Senna montana*

Furthermore, ethnobotanical claims from the Indian subcontinent are now being validated through advanced metabolic profiling, confirming that crude extracts from leaves, bark, and seeds possess a heterogeneous array of therapeutic leads (Iweala *et al.,* 2023; Fanoro & Oluwafemi, 2025). The systematic evaluation of these traditional drugs remains a critical frontier in discovering "ideal" antibiotics that are both effective and biologically sustainable (Iyswarya *et al.,* 2026; Ullah *et al.,* 2024). *Senna montana* Pod remains scientifically under-characterized with sparse literature regarding its pharmacological properties. Despite its traditional and ecological significance, this study seeks to address systematic biochemical profiling of pods secondary metabolites and evaluating its antibacterial therapeutic potential.

Materials and Methods

Senna montana fresh fruits (Pods) were collected, washed thoroughly and shade dried. Further, pods were macerated with organic and inorganic solvents followed by solvent extraction methods like maceration (Alemu *et al.,*2015; Singh *et al.,* 2022).

All the chemicals used in the study were of Analytical grade. Solvents and Specific diagnostic reagents, Sigma-Aldrich, HiMedia Laboratories (Mumbai, India) and specialised kits (Universal Biotechnology, India & Elabscience, India) which were procured from SSR Scientifics, Tirupati, Andhra pradesh. Antibiotics (Amoxicillin- Novamox, Doxycyclin- Doxy-1 L-B) were obtained from Cipla Ltd.

Phytochemical screening

Preliminary phytochemical evaluation of all the extracts was performed by following standard methods of Harborne (1973,1998) and modified protocol of *Savithramma et al.*, (2011a,b,c,d). Followed Sasidharan *et al.,* (2011**)** and Ramakrishna and Savithramma (2023) protocol for using multiple solvents.

Antibacterial Assay

Bacterial Strains and Inoculum

Pure bacterial strains used for the study were procured from the Nexus Lifesciences, Guntur. The testing strains were Gram Positive (*Staphylococcus aureus and Bacillus subtilis*) and Gram negative*, (Klebsiella pneumoniae, Escherichia coli)* and were cultures in Mueller-Hinton Broth (MHB) at 37^0c. The bacterial inoculum was standardized to the 0.5 McFarland turbidity standard ($1.5X10^8$ CFU/mL.).

Senna montana fresh Pods were dissolved in DMSO to prepare various concentrations. Positive control were Amoxicillin and Doxycycline. DMSO as negative control.

The antibacterial activity of *S.montana* Pods was evaluated using Agar well Difusion method (Khatri *et al*.,2023). Mueller-Hinton agar plates were inoculated by spreading 100 µL of the bacterial suspension evenly. Wells of 6mm diameter were bored into the agar using a sterile cork borer. Loaded with different concentrations of the pod extracts (25, 50, 75, 100 mg/ mL) and reference antiobiotic in one well (5 µg/ mL) using micropitte. The plates were left 3 hours as pre-diffusion time and incubated at 37ºC for 18-24 hours. Further, the diameter of the zones (ZOI-Zone of inhibition) is measured with Antibiotic zone scale-C (HiMedia) (Balouiri *et al*.,2016; Al-Huqail *et al*.,2020).

Results and Discussion

The qualitative phytochemical analysis of *S. montana* pod extracts, performed across a solvent polarity gradient (Water, Ethanol, Ethyl Acetate, and n-Hexane), is summarized in Table 1. The screening revealed a diverse array of secondary metabolites, with their distribution significantly influenced by the extraction solvent's affinity.

Based on the result, comparative analysis of the four solvent systems (Table.1), the biochemical profile of *S. montana* pods demonstrated across a polarity gradient reveals a distinct partitioning of bioactive metabolites, where the high-polarity fraction (Water and Ethanol) serves as the primary reservoir for Anthraquinone glycosides, Phenolics, and Tannins. Contrariwise, the non-polar n-hexane extract selectively isolated the lipid-rich seed matrix, considered by a dominance of Phytosterols and Fixed Oils, while the intermediate Ethyl Acetate fraction exclusively concentrates the Resin content. In water and ethanol, widest variety of bioactive molecules were identified (Phenols, Flavonoids, Tanins, Anthraquinones, Sugars) In Aqueous extract, Gums, Mucilage, Phytic acid were found due to their high water solubility. Non Polar etract (n-Hexane showed Alkaloids. Phytosterols, Oils and steroids, Uniquely

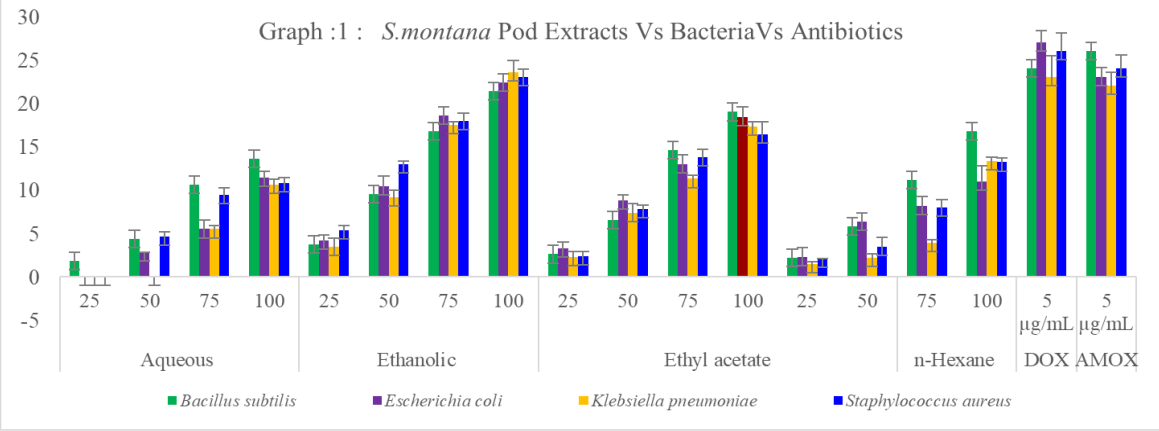
Cyanogenic glycosides as well as lignins were present. It was reported that *Senna* Pods posses Sennosides and posses medicated properties which reduce abdominal cramping (Franz, 1993; Brunton *et al*., 2018). Vasavada and Chauhan, (2023) performed statistical optimization and antimicrobial screening of Fabaceae plant extracts. Khatri and Rana (2022) demonstrated Comparative antibacterial potential of *Cassia* species pods and standard antibiotics against human pathogens.

The significant antibacterial activity observed in *S.montana* ethanolic extracts aligns with the findings of Vasavada and Chauhan, (2023) established a framework for the statistical optimisation of Fabace species screening. Furthermore, the concentration-dependent inhibition recorded in this study also aligns with the study conducted by Khatri and Rana (2022), where Cassia species Pod derived extracts demonstrated antibaterial capacity.

Table.1 Preliminary Phytochemical screening of *Cassia montana* Pods.

Secondary Metabolite	Universal solvent	Polar	Intermediate	Non Polar
	Water	Ethanol	Ethyl Acetate	n-hexane
Alkaloids	+	+	-	+++
Flavonoids	++	+++	++	+
Anthocyanidins	+	++	-	-
Anthraquinones	+++	+++	++	-
Reducing Sugars	++	++	-	-
Phenolic Compounds	+++	+++	++	-
Mucilage	+++	+	-	-
Saponins	+++	++	-	+++
Tannins	+++	+++	+	++
Cardiac Glycosides	-	-	-	++
Cyanogenic Glycosides	-	-	+	++
Phytosterols	-	-	+	+++
Fixed Oils/Fats	-	-	+	+++
Steroids	-	-	++	+++
Lignins	-	-	-	+
Gums	+++	-	-	-
Resins	-	+	+++	++
Phytic Acid	+++	+	-	-
Oxalates	+++	+	-	-

Graph.1 Antibacterial activity of *Senna montana*



Antibacterial activity

The antibacterial potential of *S.montana* Pods extracts (Aqueous, Ethanolic, Ethyl Acetate, and n-Hexane) against the selected bacterial strains (*Bacillus subtilis, Staphylococus aureus, Escherichia coli* and *Klebsiella pneumoniea*) for the susceptibility which showed varied substantial antimicrobial activity. The results, as illustrated in Graph 1, reveal that all extracts possessed varying degrees of inhibitory activity, which was markedly influenced by both the solvent polarity and the extract concentration increased from 25 mg/mL to 100 mg/mL, there was a linear increase in the ZOI across all solvents (Graph 1).

At the high concentration (100 mg/mL) the ethanolic extract displayed most potent activity, ZOI's ranging from 21mm to 23 mm and when statistically compared with DOX and AMOX yielded ZOI's of approx. 23-27 significantly lower concentration (5 µg/ mL). Across most extracts, *Bacillus subtilis* and *Staphylococcus aureus* showed higher sensitivity when compared with *Klebsiella pneumonia*. The antibacterial potency in the descended order : Ethanolic extract > n-Hexane extract >Aqueous extract. Where Aqueous extract showed negligible activity at lower concentration (25-50 mg/mL). Studies (Cushnie and Lamb 2021; Vaou *et al.,*2021) justified the comparision of high dose extracts to lowest dose pure antibiotics.
Statistical evaluation using a two way ANOVA followed by Tukey's HSD post-hoc test revealed significant differences (($p < 0.05$), in the zones of inhibition between various Pod extracts and the standard antibiotics across tested bacterial strains.

When comparing the extracts specifically against the standard antibiotic Doxycycline (DOX), a series of Student's t-tests highlights a remarkable performance by the Ethanolic extract. At the 100 mg/mL concentration, the Ethanolic extract achieved ZOI values (ranging from 21.0 to 23.5 mm) that approach the standard set by DOX (approx. 24.0 to 27.0 mm). Specifically, for *K. pneumoniae*, a t-test shown a non-significant difference ($p > 0.05$) between the 100 mg/mL Ethanolic extract and the 5 µg/mL DOX control.

A statistical comparison against Amoxicillin (AMOX) further underscores the potency of the polar extracts. While the Aqueous and n-Hexane extracts remained statistically inferior to AMOX across all bacterial strains ($p < 0.01$), the Ethanolic and Ethyl Acetate extracts demonstrated competitive results. For *S. aureus*, the ZOI of the Ethanolic extract (~23.0 mm) shows no statistically significant deviation from the AMOX control (~24.0 mm).

In conclusion, the preliminary phytochemical screening and antibacterial analysis of *Senna montana* pod extracts reveal a high concentration of bioactive secondary metabolites with significant potent therapeutic potential. The ethanolic extract emerged as the most efficient solvent for extracting therapeutic constituents demonstrating good antibacterial efficacy across all tested pathogenic strains, displaying a clear concentration-dependent inhibitory profile.

The broad-spectrum activity of the extract, particularly its effectiveness against *E. coli*, mirrors the pharmacological trends observed in the reference standard Doxycycline. Furthermore, its inhibitory action against Gram-positive strains, such as *B. subtilis* and *S. aureus*, aligns with the performance of Amoxicillin. These findings suggest that the *S. montana* pods contain a synergistic complex of multiple phytocompounds capable of targeting diverse bacterial mechanisms. Consequently, *Senna montana* Pods represents a compelling candidate for further bioactive isolation and characterization in the development of novel natural antimicrobial agents.

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Author Contributions

P. Sudhakar -Research Investigation, analysis, writing draft; S.Ankanna – Methodology, Experimentation; N.Savithramma- Reviewing, Concept.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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