Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
Impact Factor (RJIF): 6.35
www.phytojournal.com
JPP 2025; 14(5): 525-529
Received: 08-07-2025
Accepted: 11-08-2025

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Antimicrobial effect of leaf extract and mother tincture of *Senna sophera*

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DOI: https://www.doi.org/10.22271/phyto.2025.v14.i5g.15614

Abstract
Senna sophera is reported to be used in Ayurveda, Unani, folk medicine and homoeopathy for treatment of a number of clinical conditions such as asthma, bronchitis, skin infections, diabetes etc. In the present investigation an attempt has been taken to study the antifungal and antibacterial activity of ethanolic leaf extract and homoeopathic mother tincture of *Senna* against *Aspergillus niger*. Qualitative and quantitative phytochemical analysis of leaf extract showed the presence of alkaloid, tannin, anthraquinone glycoside, protein, carbohydrate, saponins, steroids, terpenoids, high concentrations of phenols, flavonoid and antioxidant activity. *Senna* leaf extract demonstrated strong inhibitory effect on radial growth of *A. niger* in a concentration-dependent manner in solid PDA medium. Highest inhibition rate of 78.75% was noted at 40% concentration. The concentration of 300 ppm *Senna* mother tincture resulted in complete inhibition (100%) of mycelial growth. Significant inhibition was also noted at 100ppm and 200ppm concentrations. Antibacterial assay with *Senna* leaf extract demonstrated greater inhibitory effect (1.4-fold) against *E. coli* than *Bacillus* sp. Docking studies of gallic acid and quercetin with Farnesyltransferase (FTase) from *Aspergillus fumigatus* showed strong interaction of amino acids of the enzyme with the polyphenols. Present study revealed a great potential to utilise this medicinal plant for development of novel antimycotic drug.

Keywords: *Senna sophera*, *Aspergillus niger*, antifungal, antibacterial, Farnesyltransferase

Introduction
Senna sophera (L.) Roxb, commonly known as *Senna*, is an erect shrub of Caesalpinaceae, found in India and tropical regions. The native origin of this plant is in tropical America [1]. The plant is known by its vernacular name in various parts of India viz. Kasondi (Hindi), Kasamarda (Sanskrit), Junglitakla (Marathi), Ghodachakumda (Oriya), Kalkasunda (Bengali), Ponnavarai (Tamil), Thounam (Manipuri) etc [2].

In India more than 8000 plants are reported to be used in traditional and herbal medicine as per Botanical Survey of India. (Press information of Bureau, Govt of India, 2022) [3]. Medicinal plants have been utilized extensively to produce novel therapeutic and preventive medicines against various diseases [4]. *Senna sophera* is reported to be used in Ayurveda, Unani and folk medicine in the treatment of asthma, bronchitis, allergic rhinitis ringworm infection, skin infection, diabetes, piles, jaundice, fever, rheumatoid arthritis, joint pain, gastrointestinal diseases and epilepsy [5-8]. Different Homoeopathic literature suggests that mother tincture produced from fresh leaves and seeds of *Senna sophera* is used in treatment of clinical issues related to the respiratory system and skin like acute and chronic asthma, eczema, psoriasis and dermatitis [9]. Besides its medicinal property, leaves and pods of this plant are edible. In Bengali ritual, the leaves of this plant are eaten as a component of "choddo shaak" (fourteen vegetable greens) on the auspicious day before Kalipuja.

Aspergiillus species are filamentous fungi which are commonly present in soil and are also found on a variety of substrates in different environment. *Aspergillus* infection and allergy may cause detrimental effects on human health resulting into serious health hazards [10, 11]. According to the official website of allergens (www.allergen.org), several species of *Aspergillus* such as *A. fumigatus*, *A. niger*, *A. flavus*, and *A. oryzae*, are known to be allergic. Till date 21 known and 25 predicted allergens were identified from the most fatal *Aspergillus fumigatus* strains [12]. There is a close association between alarming incidence of asthma and high concentration of spores of *Aspergillus sp* [13, 11].

In the present study an attempt has been taken to study the antifungal activity of *Senna sophera* leaf extract and mother tincture against *Aspergillus niger*.

Materials

Plant material and fungal culture: Plants of *Senna sophera* were collected from the surrounding locality. The culture of *Aspergillus niger* was collected from Mycopathology laboratory, Scottish Church College. The culture was maintained in PDA (Potato Dextrose Agar) medium at 30±2°C (Fig. 1&2).

Fig 1: Leaves of S*enna sophera* plant

Fig 2: *Aspergillus niger*

Methods

Preparation of plant extract and mother tincture: To prepare the plant extract[14], the leaves were collected, washed and sun dried. The dried materials were then finally grinded to powder, sealed in air tight container and stored away from light and moisture until used for extraction. A total of 50 grams of powdered leaves was mixed with 200 ml of a solvent blend made by combining ethanol and distilled water in equal proportions (1:1, v/v). This mixture was placed in dark-coloured bottles and agitated continuously on a shaker for 72 hours. After this, the mixture was initially strained through muslin cloth and then further filtered using Whatman filter paper No. 1 into a clean flask. To remove the ethanol content, the filtrate was gently heated in a water bath at 60 °C for 30 minutes. The resulting extract was stored in dark and kept refrigerated at 4 °C for preservation. A commercially prepared mother tincture of *Senna sophera* (Q) was obtained from a local homoeopathic pharmacy.

Earlier pilot experiments were performed with methanolic and aqueous extracts. Since ethanolic extract showed the highest rate of inhibition, further experiments were carried out with the ethanolic leaf extracts. The antifungal assay with ethanolic extract of *Senna* leaf demonstrated a strong inhibitory effect against *A. niger* radial growth in a concentration-dependent manner showing the highest inhibition rate of 78.75% at 40% concentration. Significant inhibition in radial mycelial growth was observed in all concentrations from 10-40%. Results of antifungal assay revealed that *Senna* leaf extract showed significant suppression of fungal growth in solid medium with greater inhibition observed at higher concentrations. (30 and 40%) (Fig.3). About 50% inhibition in mycelial growth was observed with 20% leaf extract in liquid medium. The antifungal assay with mother tincture of *Senna* leaf in solid (Fig 4.) and liquid medium (Fig. 5) also demonstrated significant inhibitory effect against *A. niger*. In the control set the fungal biomass showed the highest mycelial growth with no inhibition. As the concentration of the tincture increased, mycelial growth decreased gradually. The concentration of 300ppm *Senna* mother tincture resulted in the complete inhibition (100%) of mycelial growth. Significant inhibition was also noted at 100 ppm and 200 ppm.

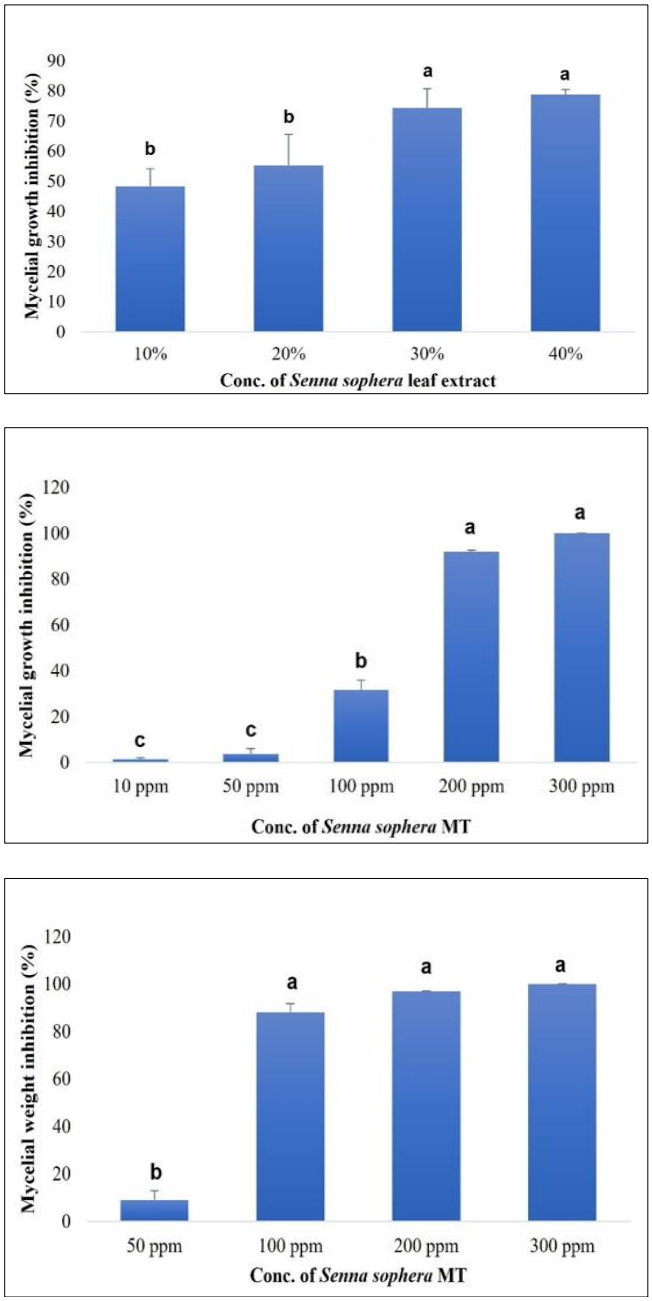

Fig. (3-5): Mycelial growth inhibition of *A. nig*er by *Senna* leaf extract and mother tincture (MT).

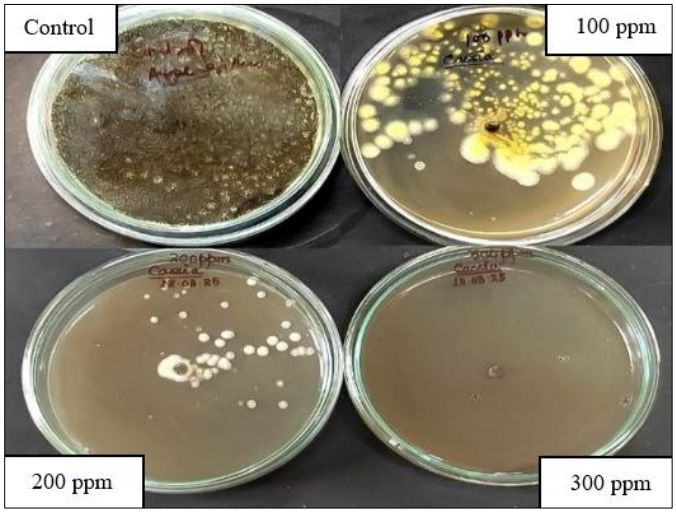

Fig 6: Mycelial growth of *A. niger* in different concentrations of *Senna* MT

Phytochemical analysis: Qualitative tests for Alkaloids, Flavonoids, Carbohydrates, Saponins, Steroids, glycosides, Terpenoids, tannins, proteins, amino acids and quantitative phytochemical analysis of Phenol, Flavonoid, Protein and antioxidant activity were carried were done following the standard methods [15-19].

Antifungal Assay: Antifungal assay with *Senna sophera* leaf extract and *Senna sophera* mother tincture was carried out both in solid (PDA) and liquid (PDB) medium by Poisoned food technique [20], using varying concentrations of leaf extract and mother tincture. The inhibitory effect of the extract and tincture was assessed by measuring the radial growth of the fungal colony in solid medium. The results were compared to control plates using the following formula:

$I = (C-T)/C \times 100$

Where 'I' refers to percentage of inhibition, 'C' is control, and 'T' is radial growth of fungus (cm) in treated sets [20].

In liquid medium the dry weight of the mycelial growth was measured and percentage of inhibition of mycelial growth with respect to control was measured.

Antibacterial Assay: Antibacterial assay of leaf extract was carried out with *Bacillus sp* and *Escherichia coli* by well diffusion assay technique. Luria Bertani agar medium was prepared and poured in Petri plate. Bacterial inoculum was then applied on the plate by spreader and wells were cut onto the agar plate. Leaf extract of *Senna* (60µL) was then added in each of the wells and the diameter of zone of lysis was measured after incubation of 24 hours.

Molecular Docking: Molecular modelling studies were performed to investigate binding of the polyphenols to Farnesyl transferase. For docking study of gallic acid and quercetin with farnesyltransferase, the crystal structures were obtained from PDB structural data base. Docking studies were performed with Auto Dock 4.2 software, which calculated the interaction between the ligands and the enzyme. The probable conformers of the ligands that bind to the enzyme were obtained using the Lamarckian genetic algorithm. For the docking process, a maximum number of 50 conformers were considered for ligand binding to the protein, and the lowest binding-energy conformation was chosen for further analysis. The lowest energy binding domain was visualized using PyMOL Molecular graphics system.

Results

The ethanolic extract of *Senna* leaves revealed the presence of a wide range of bioactive phytochemicals such as alkaloids, flavonoids, tannins, carbohydrates, saponins, steroids, anthraquinone glycosides, terpenoids, proteins and amino acids. The broad spectrum of secondary metabolites detected supports the traditional use of *Senna sophera* in herbal medicine and highlights its potential for therapeutic applications. The leaf extract showed high flavonoid and total phenol content indicating rich presence of natural antioxidant compounds. Additionally, the extract exhibited high antioxidant activity in DPPH assay, demonstrating strong free radical scavenging ability. (Table 1&2)

Table 1: Phytochemical analysis of leaf extract of *Senna*

Phytochemical Test	Ethanolic Extract of *Senna sophera*
Alkaloid	+
Flavonoid	+
Tannins	+
Carbohydrates	+
Saponins	+
Steroids	+
Anthraquinone glycosides	+
Cardiac glycosides	-
Terpenoids	+
Gum & Mucilage	-
Proteins & Amino acids	+

Table 2: Phenol, flavonoid, protein content and antioxidant activity in leaf extract of *Senna*

Phenol (µg/g)	Flavonoid (µg/g)	Protein (mg/ml)	DPPH (%)
1946.02	5991.16	25.68	77.4

Antibacterial assay of *Senna* leaf extract demonstrated a differential inhibitory effect against the tested bacterial strains. The zone of lysis was greater (1.4-fold) in *E. coli* than *Bacillus* sp. The result showed a positive approach of antibacterial effect of *Senna* leaf extract against gram negative bacteria.

Table 3: Antibacterial effect of *Senna* leaf extract.

Sl. No.	Bacteria	Mean diameter of zone of lysis (cm)
1.	*Bacillus sp*	1.03±0.04
2.	*E. coli*	1.40±0.30

Docking studies of the gallic acid and quercetin with Farnesyltransferase (FTase) from *Aspergillus fumigatus*, provided some insight about the binding mode of the compounds, which in turn can be correlated with the enhanced activity of the enzyme, FTase (PDB ID - 4MBG). The negative binding energies values (-7.38 for Gallic Acid and -8.2 for Quercetin) derived from Autodock indicate favourable binding interactions of the enzyme with the polyphenols. In the docked conformation of both gallic acid and quercetin with the FTase, a couple of polar neighbours are found surrounding them. These polar contacts can justify well the existence of the hydrogen bonding interactions. However, couple of hydrophobic residues is found close to quercetin in their respective docked conformation with FTase. The presence of these hydrophobic residues indicates hydrophobic interaction in the complexation process. The docked pose of the polyphenols showing interactions of amino acid residues of the enzyme is shown in Fig 7 & 8 and Table 4 &5.

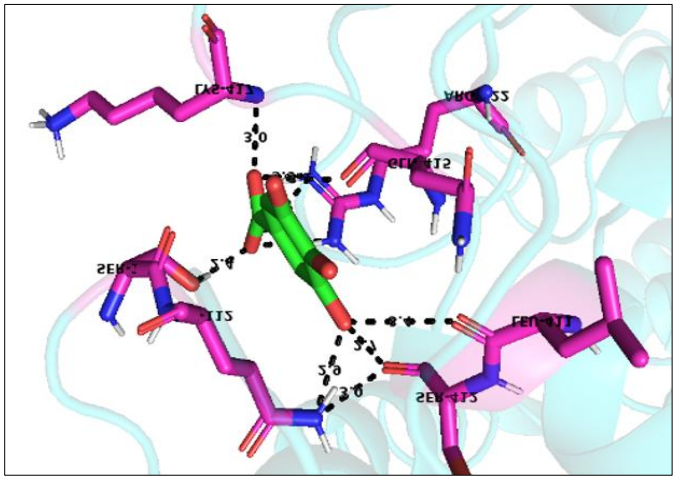

Fig 7: Docked pose showing interactions of amino acid residues of the enzyme with Gallic acid

Table 4: Interacting amino acid residues of Farnesyltransferase with Gallic Acid

Interacting amino acid residues	Distance (in Angstrom)
Ser111	2.4
Gln 112	2.7, 2.9
Leucine (L) 411	3.4
Serine (S) 412	2.7, 2.9, 3.4
Lysine (K) 417	3
Glutamine (Q) 415	3.1
Arginine (R) 422	3.0, 3.0, 3.5

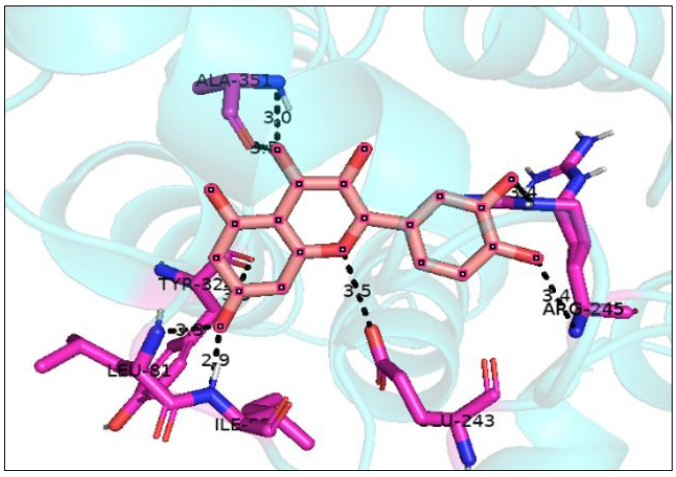

Fig 8: Docked pose showing interactions of the amino acid residues of the enzyme with Quercetin.

Table 5: Interacting amino acid residues of FTase with Quercetin

Interacting amino acid residues	Distance (in Angstrom)
Leucine (L) 81	3.3
Isoleucine (I) 82	2.9
Glutamate (E) 243	3.5
Arginine (R) 245	3.4
Tyrosine (Y) 324	3.3
Alanine (A) 351	3.0, 3.5

The genus *Aspergillus*, which consists of a few hundred opportunistic mold species are well known to cause health hazards such as allergy and infection. Several species of *Aspergillus* are noted for their allergic property in human.[11] Among the species, *Aspergillus fumigatus* is the most common and life-threatening airborne opportunistic fungal pathogen particularly infecting the immunocompromised hosts.[21] Present investigation was carried out with *A. niger* which is also reported to be an opportunistic pathogen and an allergen. Since *S. sophera* has been reported as an established ethnomedicine and homoeopathy drug that can cure asthma and other clinical conditions related to respiratory system, the drug might also be effective against the allergens of *Aspergillus* spp. Further molecular docking study showed good binding of FTase with polyphenols gallic acid and flavonoids. FTase is reported to be essential for growth and virulence in several pathogenic fungi and therefore it can be an attractive target for the discovery of new antifungals. Since there is a dearth of therapeutics to treat fungal infections in humans, this study may have immense potential in the field of development of antifungal drug using medicinal plant.

Conclusion

Present study revealed the antifungal and antibacterial property of *S.sophera*. Although the plant is known as a potent traditional and a homoeopathic drug, this investigation further highlights the efficacy of this plant as a potent antifungal agent against *A.niger*. Further extensive research in this field is needed to be carried out to design new antimycotic drug against the allergens of *Aspergillus* species.

Acknowledgement

The authors acknowledge DST-FIST Programme, Govt of India [SR/FST/College-/2023/1486
(G)] for providing infrastructure facilities in the college.

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