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Phytochemical and Antimicrobial Screening of *Senna italica* Mill. Leaf

S. Menaga*, G. Jayanthi, P. Haritha and Naveena

PG and Research Department of Botany, Vellalar College for Women (Autonomous), Erode – 638012, Tamil Nadu, India; menaga.dhanu@gmail.com

Abstract

The use of medicinal plants has gained more importance because of their natural origin and high therapeutic significance. The bioactive compounds detect of methanol and aqueous from the leaves of *Senna italica* showed non-ketone compounds, glycosides, terpenoids and steroids. The ongoing work scope us to study the impact of methanol and watery leaf juice on *Senna italica* with against bacteria (Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli*) and fungal (*Aspergillus niger, Aspergillus flavus*) activity. In general, among the extracts showed methanol reveals higher inhibition than with watery extracts. However, it was evidence that aqueous extract. It was evident that at higher concentrations magnitude the zone of inhibition in higher level.

Keywords: Antimicrobial Activity, Fabaceae, Phytochemical Analysis, *Senna italica*

1. Introduction

Plant herbs are the gift of nature to mankind and they make disease less healthier lifestyle. Because living flowering crops possess miraculous and dangerous power, which could alleviate pain and also cure of illness. Despite recent development in synthetic drug discoveries, plants still occupy an important place in the modern and ancient systems of drugs all over the world. Medicinal plants, which constitute a segment of the flora, prepare the raw material for use in all the local systems of medicine in India includes Arab and other systems of India medicines and Tibetan drug (Das, 2008). The WHO declared 80% of the world's population rely primarily towards ancient medicines for their main healthcare. Plant dependent products also play a vital role in the system health and remaining 20% depends on residents.

The genus *Senna* and its species are used to treat ailments such as intestinal complications, hemorrhoids and circulatory disorders. A variety of bioactive compounds found in plants are useful in human and veterinary medicines (Sajal *et al*., 2014). So an analytical work in this line is necessary to analyse the bioactive constituents and antimicrobial activities of the common plant namely *Senna italica* under the family Fabaceae.

2. Materials and Methods

2.1 Plant Collection and Identification

Senna italica leaves were collected in November 2019 from Anthiyur, Erode District. With the help of "Hebarium Specimen available in Department of Botany, Vellalar College for Women.

2.2 Preparation of Plant Extracts

The collected plant materials were washed with tap water and dried under shade condition. The air dried leaves were ground into a coarse powder (Plate -1). For methanol extraction the Soxhlet apparatus was used and in the case of aqueous extraction cold perculation method was followed. About 20 gm of dried powder was packed with thimble and then subjected to be methanol extraction. The collected extract was concentrated by evaporation at room temperature and was stored for future analysis.

Common Names

English	:	Ishrig
Tamil	:	Nilaavarai
Malayalam	:	Seruvanni, Sunnamaki
Telugu	:	Nelaponna, Nelathangedu
Kannada	:	Adavithangadi, Kaadu Sonnaamukhi

**Author for correspondence*

(A)

(B)

Plate 1. **(A)** Habit of Senna italica **(B)** Coarse powder of Senna italica.

2.3 Preliminary Phytochemical Analysis

Methanol and aqueous extracts were subjected to preliminary phytochemical tests followed by the methods of Horborne (1984), Trease and Evans (1989), Kokate *et al.* (1995) and Prabhakaran (1996). Both the extracts were screened to confirm the presence of bioactive constituents like carbohydrates, proteins, alkaloids, phenols, tannins, flavonoids, aldehydes, glycosides, steroids, saponins and anthroquinones by using following tests. The tests were based on the visual observations of colour change or formation of precipitate after addition of specific reagents.

2.4 Priliminary Phytochemical Studies

2.4.1 Test for Carbohydrates (Anthrones Test)

3 ml of sample was tested with 5 ml Anthrone reagent which showed formation of yellow color indicates the presence of carbohydrates.

2.4.2 Test For Proteins (Ninhydrin Test)

Ninhydrin was added to the 3 ml of test sample and boiled for few minutes. The presence of the compound was indicated by the formation of blue color.

2.4.3 Test for Alkaloids (Wagner's Test)

To 1 ml of test solution few drops of Wagner's reagent was added. The formation of yellow precipitate indicates the presence of alkaloids.

3. Test for Tannins (Ferric Chloride Test)

A few drops of 0.1% of ferric chloride was added to the 1 ml of test solution. The presence was indicated by brownish green or a blue color.

4. Test for Flavonoids (Alkaline Test)

To 2 ml of test solution, 2 ml of 10% NaOH was added. Intense yellow color was formed which disappears on addition of dilute HCl. This shows the presence of flavonoids.

5. Test for Terpenoids (Salwokski Test)

2 ml of chloroform was added to the 3 ml of test solution and then 3 ml of con. H_2SO_4 was added. A reddish brown color in interphase indicates the presence of terpenoids.

6. Test for Steroids (Salwokski Test)

To 3 ml of test sample, 1 ml of chloroform and equal volume of H_2SO_4 was added to the side of the tube. The upper layer turns red and lower layer turns yellow with green fluorescence which indicates the presence of steroids.

7. Test for Saponins (Foam Test)

Add 2 ml of water to the test solution and shake well. Stable foam for 10-15 minutes indicates the positive result for saponin.

8. Test for Glycosides (Acetic Acid Test)

Glacial acetic acid and ferric chloride was added as drop to the test solution to that conc. H_2SO_4 was added. A reddish brown color for the indication of positive result.

9. Test for Anthroquinones

To 0.5 ml of extract was added with a few drops of HCl. The mixture was taken and appearance of pink, red or violet color in the lower phase indicates the presence of anthroquinones.

10. Antimicrobial Activity

The tested microbial strains were collected from the Department of Biotechnology, KSR College of Technology, Tiruchengode, Namakkal District, Tamil Nadu.

11. Preparation of Inoculums

The bacterial organisms like Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli*) and the fungal organisms (*Aspergillus niger* and *Aspergillus flavus*) were pre cultured in nutrient broth overnight. Bacterial cultures were grown in Muller Hinton Agar medium and the fungal cultures are grown in Potato Dextrose Agar medium and these inoculums were used for antimicrobial assay.

12. Composition of Medium

Muller Hinton Agar medium

Beef infusion	:	300 g
Acid hydrolysate of Caesin	:	17.5 g
Starch	:	1.5 g
Agar	:	17 g
Distilled water	:	1 Lit.

Potato Dextrose Agar medium

Potato infusion	:	200 gm
Dextrose	:	20 gm
Agar	:	20 gm
Distilled water	:	1 liter

13. Antimicrobial Assay

The above nutrients were weighed and dissolved in water. To dissolve the agar the mixture was warmed on water bath at 15 lbs pressure, 121°C sterilized in an autoclave for fifteen minutes. The sterilized medium (20 ml) was poured into a sterilized petriplates under aseptic condition, allowing them to solidify. The plant extracts were tested against *Escherichia coli, Staphylococcus aureus, Aspergillus niger* and *Aspergillus flavus* by using the agar well diffusion assay for antimicrobial activity.

14. Agar Well Diffusion Assay (Bauer *et al.*, 1968)

The plant extracts were tested for antibacterial and antifungal activity. The Agar well diffusion method was employed for the determination of antimicrobial activity of the methanol and aqueous extracts. The bacterial cultures were inoculated in nutrient broth and the fungal strains were inoculated in PDA broth at 37°C for overnight. The Prepoured plates were inoculated by dipping sterile swab into inoculums. The excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube. The swab was streaked well all over the surface of the medium, rotating the plate through an angle of 60°C after each application. Finally, the swab was passed around the edge of the agar surface then closed with the lid. The inoculation was dried for a few minutes at room temperature. With the help of cork borer put a well in the place at a regular interval. Add standards Chloramphenicol (30 mg) as standard used for bacteria and Miconazole (30 mg) as a standard for fungi and extracts in different concentration (20 µg, 40 µg and 60 µg) in the well. Within in 30 minutes, the plates were incubated at 37°C for bacteria and 22°C for fungi. After incubation approximate period for bacteria is two days and 7 days for fungi. The zone of diameter of inhibition (including the diameter well) was measured and recorded in millimeter (mm). The measurements were taken with a ruler, from the bottom of the plate, without opening the lid.

15. Results and Discussion

The bioactive constituents of *Senna italica* were evaluated both methanol and aqueous extracts by using qualitative

phytochemical tests. The presence was expressed as (+) and the absence was expressed in (-) symbol. The antimicrobial activities of *Senna italica* was tested against bacteria like *Escherichia coli* and *Staphylococcus aureus* and against fungi like *Aspergillus niger* and *Aspergillus flavus* by measuring the diameter of zones of inhibition.

16. Qualitative Phytochemical Analysis of *Senna italica*

The phytochemical screening of methanol extract from the leaf of *Senna italica* showed the presence of phytochemical constituents such as carbohydrates, proteins, flavonoids, alkaloids, glycosides, terpenoids and steroids. At the same time, the bioactive constituents like tannins, saponins and anthroquinone were totally absent. The results obtained from the qualitative phytochemical studies are given in Table 1.

Table 1. Qualitative phytochemical screening of methanol and aqueous extract of *Senna italica* mill

S. No.	Phytochemical constituents	Name of the Extract	
		Methanol	Aqueous
1.	Carbohydrates	+	_
2.	Proteins	+	_
3.	Alkaloids	+	+
4.	Tannins	_	+
5.	Flavonoids	+	+
6.	Terpenoids	+	+
7.	Steroids	+	_
8.	Saponins	_	+
9.	Glycosides	+	+
10.	Anthroquinone	_	_

Note: + = Positive, - = Negative

Parallel results observed in the bioactive findings with the extracts of methanol in *Senna italica* leaves were obtained by (Dabai *et al.,* 2012). Vijaya Bharathi *et al.,* 2018 reported the presence of phytoconstituents like steroids, flavonoids, glycosides, tannins and carbohydrates from the leaves and seed extracts of *Senna italica*. This is in accordance with the present investigation. The medicinal plant species of *Senna italica* was screened to detect the presence of several bioactive compounds which are reported to cure different diseases and ailments. Same active principles have also been reported by (Bhalerao and Kelkar, 2012; Dabai *et al.,* 2012 and Gollo *et al.,* 2016). Presence of these compounds can be correlated with the medicinal potential of the plant.

The effect of methanol and aqueous extracts of *Senna italica* were tested against two species of bacteria namely *Staphylococcus aureus* and *Escherichia coli* and against two species of fungi namely *Aspergillus niger* and *Aspergillus* using agar well diffusion method. The standards used were Chloramphenicol (30 mg) and Miconazole (30 mg).

The activity against bacteria was undertaken with *Staphylococcus aureus* and *Escherichia coli*. Methanol extract of *Senna italica* showed a higher activity against the tested bacterial organisms. And the aqueous extract of *Senna italica* also showed potent activity against both bacterial organisms. Among the two different extracts higher zone of inhibition was showed in a methanolic extract which were compared with standard Chloramphenicol and aqueous extract (Table 2, Plate 2).

The antifungal activity was undertaken against *Aspergillus niger* and *Aspergillus flavus*. Methanol and the aqueous extract of *Senna italica* showed a potent activity against both fungal organisms. Among the two extracts more inhibitory zone was showed in a methanol extract when compared with standard Chloramphenicol and aqueous extract (Plate 3, Table 3).

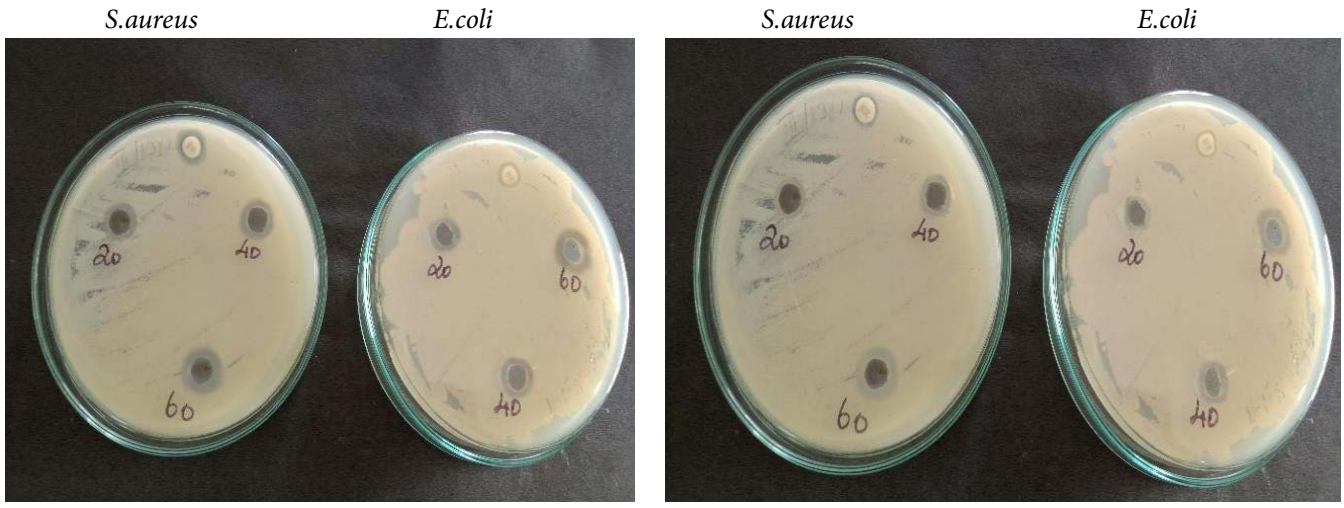
The presence of the identified phytochemical components makes the leaves medicinally active. In the proximate analysis the leaf nutrients in the plant that are useful for many pharmacological activity. The antibiotic

Table 2. Antibacterial activity of *Senna italica* leaf extracts by agar well diffusion method

S.No.	Name of the organism	Name of the Extract								
		Methanol			Aqueous			Standard Chloramphenicol		
		20 µg	40 µg	60 µg	20 µg	40 µg	60 µg	20 µg	40 µg	60 µg
1	*Staphylococcus aureus*	0.7	0.8	0.9	0.5	0.5	0.7	1.2	2.2	2.2
2.	*Escherichia coli*	0.6	0.7	0.8	0.3	0.4	0.6	2.2	2.2	2.2

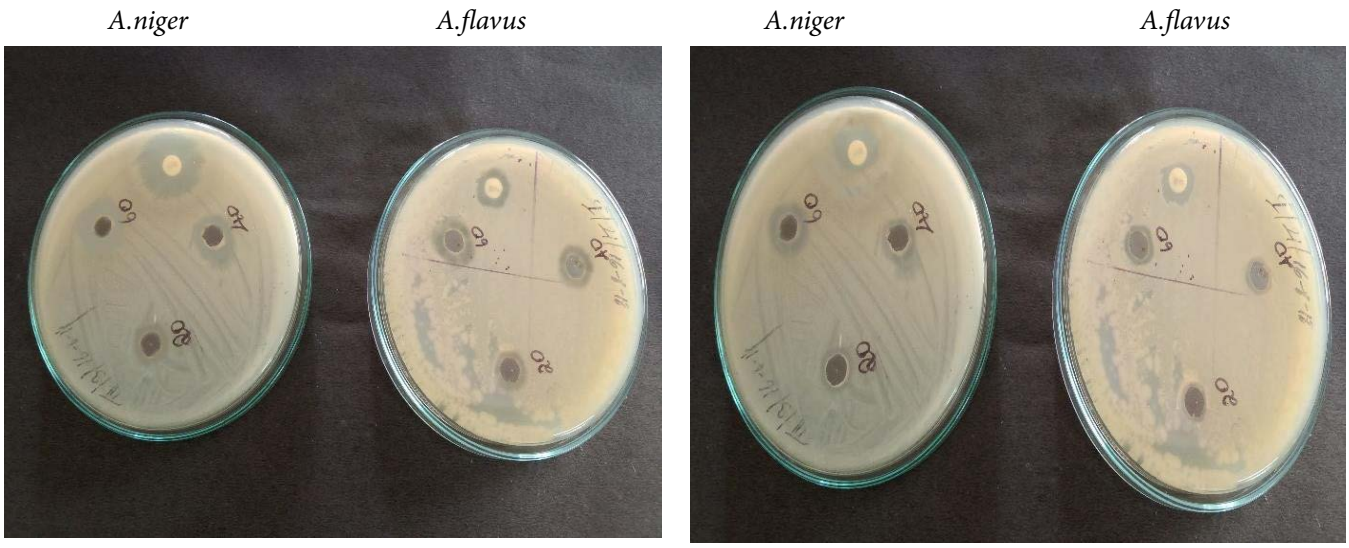
Table 3. Antibacterial activity of *Senna italica* leaf extracts by agar well diffusion method

S.No.	Name of the organism	Name of the Extract								
		Methanol			Aqueous			Standard Miconazole		
		20 µg	**40** µg	**60** µg	**20** µg	**40** µg	**60** µg	**20** µg	**40** µg	**60** µg
1	*Aspergillus niger*	0.9	0.9	1.0	0.4	0.5	0.9	0.9	0.9	0.9
2.	*Aspergillus flavus*	1.2	1.2	1.3	0.6	0.9	1.1	0.7	0.7	0.7



Methanol extract **Aqueous extract**

Plate 2. Antibacterial activity of *Senna italica* mill. leaf extracts by agar well diffusion method



Methanol extract **Aqueous extract**

Plate 3. Antifungal activity of *Senna italica* mill. leaf extracts by agar well diffusion method

resistance of pathogenic microorganisms shows continuous development and it plays a major worldwide health concern. According to (Masko *et al.,* 2010) the leaf extracts showed a range of activity against all the bacteria and fungi.

In this study, the plant extracts of both methanol and aqueous were used to evaluate its inhibitory properties on selected plant pathogens.

According to Sulieman *et al.,* 2017, methanol extract of *Senna italica* plant produce more inhibitory zones against

the bacteria like *Escherichia coli, Klebiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella* spp. Anushia *et al.,* 2009 reported that the antifungal activity against *Trichophyton mentagrophytes and Epidermophyton floccosum* and antibacterial activity against i.e., *Bacillus megaterium, Streptococcus haemolyticus and Shigella boydii* in the seed extracts of *Cassia fistula*. Sood *et al.,* 2012 studied the antimicrobial activity results against *Escherichia coli, Aspergillus niger, Aspergillus flavus, Fusarium moniliformae* and *Rhizoctonia baticola*in, *Cassia reningera*. Our findings were well coincided results obtained by above mentioned researchers.

17. Conclusion

Our presence finding reveals that the plant showing high antimicrobial activity it may be owing to the presence of above tested secondary metabolites. The studied plant extracts are having antimicrobial activity and this plant can be used as sources for identify new drugs. From this we concluded that the methanolic extract was proved to be more efficient when compared to aqueous extract. But further spectral studies are needed to standardize this study plant.

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