

RESEARCH ARTICLE

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The study area of Shervarayan Hills (Yercaud), Salem District, Tamil Nadu in Medicinal Plant of *Cassia tora* (Senna tora)(Caesalpiniaceae) in Antimicrobial Activities

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ABSTRACT

The selected study area for this study is Shervarayan Hills (Yercaud), Salem District, Tamil Nadu in Medicinal Plant of *Cassia tora* (Senna tora)(Caesalpiniaceae) in Antimicrobial Activities one of the medicinal plants. It is a tropically distributed the highly medicinal plant. Antimicrobial activities and extracts of petroleum ether, Ethyl acetate and methanol were also found to be better with respect to inhibitory function against the two fungal species, *Fusarium oxysporum* and *Aspergillus flavus*. The study scientifically validates the use of plant in traditional and ethno medicine. Three solvents such as Petroleum ether, Ethyl acetate and Ethanol were used to take plant extract. These extracts were studied for antimicrobial activity against two gram positive bacterial strains such as *Bacillus subtilis* and *Bacillus thuriengensis* and two gram negative bacterial strains such as *Klebsiella pneumonia* and *Escherichia coli*. This study also extended to find antifungal activity against four fungal strains.

Keywords: *Senna tora* (Caesalpiniaceae); Antibacterial activity: *Ethnomedicine*, *Fusarium oxysporum* and *Aspergillus flavus*; negative bacterial strains: *Klebsiella pneumonia* and *Escherichia coli*

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I. INTRODUCTION

The studies of World Health Organization (WHO) indicate that over 60% of World's plant species have been used for medicinal purposes. The medicinal value of plant is due to the presence of certain secondary metabolites. The application of plants as medicine dates back to prehistoric period. The early civilization reveals that a considerable number of drugs used in modern medicine have figured in ancient manuscripts such as the Rig, Veda, the Bible, the Quran, the Iliad, the Odyssey and the History of Herodotus. Over 700 years ago, the ancient Chinese were the first to use the plants of natural vegetation as the source of medicine. In India, in ayurvedic system of medical practice, using barks of plants have been in medicinal use for over 3000 years. Charaka and Susruta, two of the earliest Indian authors had sufficient knowledge on the properties of the Indian medicinal plants.

The medicinal plants are highly important of Human health and contributing towards the improvement of human life. Since ancient times, several plants have been used as a source of medicines. The most of varieties drugs plants could be obtained from medicines. About 85 % individuals from developing countries rely on plant based preparations used in their traditional medicinal system and as developing the basic needs

for human primary health care (Ellof, 1998). The medicine and plant derived from phytochemicals have great interest as they are the natural alternatives for synthetic compounds. Nowadays herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low cost (Valiathan, 1998).

Despite the local medicinal usage of the studied species, *Cassia tora*, no studies were carried out for confirming their medicinal uses. Therefore, in the present study, antimicrobial studies were performed to confirm their healing properties. For that, various extracts of the useful parts of this species viz., leaf, stem and root were tested against certain pathogenic bacteria and fungi. Little studies were done on this medicinal plant and no results were found regarding confirmation of their medicinal uses.

II. MATERIALS AND METHODS

Various medicinal plants in informations regarding *Cassia tora* were collected from Malayali tribes and through literature in *Cassia tora*, the leaf, stem and root are found to contain the various medicinal values and hence used for medicinal purposes. Hence, at the present study antimicrobial

properties were analyzed by using these parts against the selected microbes.

Collection and Processing of Plant Parts

Fresh leaf, stem and root parts of the study species were collected from the Shervarayan Hills (Yercaud), Salem District, Tamil Nadu. These fresh materials were washed under running tap water, air dried and then homogenized fine powder and stored in air tight bottles.

Preparation of Plant Extracts

Preparation of Plant Extracts in medicinally importance, the shade dried plant parts of the study species were made into a fine powder of 40 mesh size using the pulverizer separately. 100g of the powder was filled in the filter paper and successively extracted using 500 mL solvents viz. petroleum ether, ethyl acetate and methanol separately using the soxhlet extractor for 8 – 10 hours (Gafner *et al.*, 1985). Then the extracts were filtered separately through what man No.1 filter paper to remove the all undissolved matter, including cellular materials and other constituents that are insoluble in the extraction solvents.

Antimicrobial Activity of the Plant Extracts

The experiments were carried out to show the antimicrobial efficacy of the plant extracts to cure large number of pathogenic diseases. Antimicrobial activity of petroleum ether, ethyl acetate and methanol extracts of leaf, stem and root parts of the study species were determined by using disc diffusion method (Bauer *et al.*, 1966).

Collection and maintenance of microorganisms

The following microorganisms were used in the present study:

Bacterial strains

G (+) ve bacteria - *Bacillus subtilis* and *B. thuriensis*.

G (-) ve bacteria - *Klebsiella pneumonia* and *Escherichia coli*.

Fungal strains

Aspergillus niger, *A. flavus*, *A. baumanii* and *Fusarium oxysporum*.

These microbes were obtained from the Department of Microbiology, Kandaswami Kandar's college, Paramathi Velur, Namakkal District. The bacterial and fungal strains were maintained at 4°C on nutrient agar and potato dextrose agar slants respectively and kept in refrigerator prior to subculture.

Media used

Freshly prepared nutrient agar medium and potato dextrose agar (PDA) medium were used for the culture of bacteria and fungi respectively.

Composition of Nutrient agar medium

Constituents	Amount
Peptone	5.0g
Beef extract	3.0g
Agar	15.0g
Distilled water	1000mL
pH	7.0

Composition of PDA medium

Constituents	Amount
Potato	200.0g
Dextrose	20.0g
Agar	15.0g
Distilled water	1000mL
pH	6.2

Method

The culture mediums were prepared and autoclaved at 121°C at 15 p.s.i. for 20 minutes and stored in refrigerator. The medium were allowed to melt before the process of inoculation. The clean dry sterile Petri dishes were poured with nutrient agar medium (for bacteria) and potato dextrose agar medium (for fungus). Four numbers of 10 ml broths were prepared separately for nutrient agar medium and potato dextrose agar medium in test tubes and plugged with cotton and autoclaved. The test tubes were labelled with the microbes to be inoculated. The bacterial strains were inoculated onto the nutrient broth, and fungi were inoculated onto potato dextrose broth under aseptic conditions and incubated at $37 \pm 0.5^\circ\text{C}$ for 18 hours. After incubation, the bacteria and fungi were smeared on the nutrient agar and potato dextrose agar plate respectively using a sterile cotton swab. A sterile disc of 6 mm diameter was loaded with known quantity of 10 mg of dried crude extracts. These discs were placed on the surface of the media. The positive controls antibiotic and tetracycline were used at the concentration, 0.1 mg/10 ml of distilled water each and maintained by loading on discs. Then the Petri dishes were incubated at $37 \pm 0.5^\circ\text{C}$ for 24 to 48 hours. The diameter of inhibition zone was measured. Triplicates were maintained for all tests.

III. RESULTS

Antibacterial activity

The antibacterial activity of various extracts of the studied species was assayed *in vitro* by disc diffusion method against four bacterial pathogens. The data on bacterial growth inhibition by various alcoholic extracts of leaf, stem and root parts of studied plant species was shown in tables 18-20.

The antibacterial activity of the alcoholic leaf extracts of the study species, *Senna tora* generally showed inhibitory activity against the growth of *Bacillus thuringiensis* and *Escherichia coli* (Plate – 1a). However, towards *Bacillus subtilis* and *Klebsiella pneumoniae*, all these extracts showed activity with less pronounced manner (Table 1). The antibacterial activity of certain alcoholic stem extracts of *Senna tora* is given in Table 2. It shows that generally, all extracts have significant activity against the three bacteria viz., *Bacillus subtilis* (Plate – 1b) *B.Thuringiensis* and *Klebsiella pneumoniae* and it was less against the other bacteria, *Escherichia coli*. Similarly, the root extracts of this species has showed significant inhibitory activity against the two bacteria viz., *Bacillus thuringiensis* and *Escherichia coli* (Table 3 and Plate-1c). Further, it was noted that the inhibitory activity was noteworthy against the bacteria, *Bacillus subtilis* and *Klebsiella pneumoniae* also.

Antifungal activity

The antifungal activity of various alcoholic leaf extracts of the study species, *Senna tora* against the four studied fungal species is given in Table 4 and Plate – 1d. The results of the study report that the ethyl acetate extract has the highest inhibitory activity (14.73 mm diameter inhibitory zone) against the fungus, *Aspergillus niger*. The petroleum ether and methanol extracts were also found to be better with respect to inhibitory function against the two fungal species, *Fusarium oxysporum* and *Aspergillus flavus* (13.17 and 14.21 mm diameter inhibitory zone respectively). The petroleum ether and methanol stem extracts of this species showed greater inhibitory zone against the fungus, *A. baumannii* (Plate – 1e) (13.73 mm diameter inhibitory zone and 14.27 mm diameter inhibitory zone respectively) (Table 5). The inhibitory activity against the fungus, *Aspergillus niger* was highest (21.03 mm diameter inhibitory zone) by the ethyl acetate stem extract than the other extracts examined. The inhibition effect of alcoholic root extracts of this species is given in Table 6. The study exhibited that the ethyl acetate and methanol extracts showed highest inhibitory activity against the growth of the fungus, *Aspergillus niger* by producing 20.53 and 18.63 mm diameter inhibitory zone respectively (Plate – 1f). Petroleum ether extract showed higher inhibitory zone against the fungus, *Aspergillus flavus* (13.23 mm diameter inhibitory zone).

IV. DISCUSSION

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction

procedure. Traditional healers use primarily water as the solvent but in the present study it was found that plant extracts in organic solvents provided more consistent antimicrobial activity. These observations can be intrinsic bioactivity by their ability to dissolve or diffuse in the different media used in the assay.

The results of the present study on the antimicrobial activities of various organic chemical extracts of leaf, stem and root parts of the studied species against the colonial growth of four bacterial species and four fungal species are presented in Tables 1-6. The plant parts and solvents used for the extraction of secondary metabolites of inhibition property. For the species, *Senna tora* almost all extracts of leaf, stem and root parts in general have the considerable antibacterial activity against the four bacterial species investigated (Tables 1-3). However, the leaf, stem and root parts of the ethyl acetate and methanol extracts were found to be effective against certain specific fungi (Tables 4-6). The overall study on antimicrobial activity reports that the studied plant species containing active compounds of inhibitory action substantially. The beneficial medicinal effects of this plant materials typically results from the combinations of secondary products present in this plant species. The heterogeneity of these secondary compounds in wild species is reported to be wide (Balandrin *et al.*, 1985). Based on this concept, it is explained that this study species due to heterogeneity of secondary compounds owing to their wildness could be with higher antimicrobial activity. The higher antimicrobial activity of alcoholic extracts of the present study species may further indicates that the antimicrobial principles/chemical constituents which are either polar or non polar can be effectively extracted only through the organic solvent medium (Eseawi and Srour, 2000; Aiyelaagbe *et al.*, 2007; Rahulet *et al.*, 2011; Sumaira *et al.*, 2016). Many early studies also reported the effective inhibitory activity of alcoholic solvents against the growth of the pathogenic microbes (Thomas *et al.*, 1999; Ates and Erdogru, 2003; Salam *et al.*, 2013; SubbaLakshmi; Pullaiah, 2015; Alothyqiet *et al.*, 2016). The poor antimicrobial activity of some extracts might be attributed to the extracting capacity of solvent and the concentration of the active ingredients in the extracts (Akpomie and Olorunbon, 2011). Moreover, the effectiveness of plant extract against a particular pathogen is affected by various intrinsic and extrinsic factors (BalasahebShinde and YoginiRamkrishna, 2015).

From the present investigation, the results obtained confirm the therapeutic potency of the studied plant species of is Shervarayan Hills (Yercaud), Salem District, Tamil Nadu in Medicinal Plant of *Cassia tora* (*Senna tora*)(Caesalpinaceae), prescribed in

traditional medical practice by Malayali tribes. Further, it supports the folkloric usage of this plant and suggests that their alcoholic extracts possess compounds of activity of inhibitory and they can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobial compounds and undergo further pharmacological evaluation.

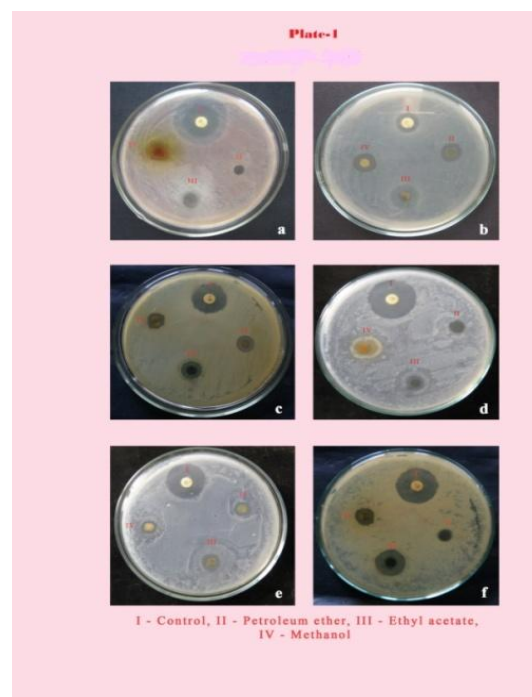


Table 1.Antibacterial activity of certain alcoholic leaf extracts of the species, *Senna tora*.

Plant extracts	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	<i>Bacillus subtilis</i>	<i>B. thuringiensis</i>	<i>K. pneumoniae</i>	<i>Escherichia coli</i>
Standard*	7.23± 0.56	6.23± 0.35	10.23± 0.44	25.19± 0.40
Petroleum	9.20 ± 0.41	8.16 ± 0.62	-	8.42 ±0.16
Ethyl	1.13 ± 0.13	11.02 ± 0.80	8.03 ± 0.15	9.31 ±0.35
Methanol	-	9.07 ± 0.21	9.61 ± 0.40	11.33 ± 0.45

* Tetracycline

Table 2.Antibacterial activity of certain alcoholic stem extracts of the species, *Senna tora*.

Plant extracts	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	<i>Bacillus subtilis</i>	<i>B. thuringiensis</i>	<i>K. pneumoniae</i>	<i>Escherichia coli</i>
Standard*	20.77± 0.20	27.16 ± 0.56	12.33 ± 0.42	16.53 ± 0.17
Petroleum ether	10.21 ± 0.16	10.16 ± 0.57	9.16 ± 0.37	-
Ethyl acetate	12.13 ± 0.61	11.03 ± 0.23	17.23 ± 0.51	13.16 ± 0.66
Methanol	11.16 ±0.47	14.17 ± 0.60	11.73 ± 0.75	9.06 ± 0.30

* Tetracycline

Table 3. Antibacterial activity of certain alcoholic root extracts of the species, *Senna tora*.

Plant extracts	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	<i>Bacillus subtilis</i>	<i>B. thuringiensis</i>	<i>K. pneumoniae</i>	<i>Escherichia coli</i>
Standard*	20.23 ± 0.49	28.17 ± 0.21	11.17 ± 0.93	22.06 ± 0.12
Petroleum ether	-	11.63 ± 0.65	-	8.93 ± 0.40
Ethyl acetate	10.87 ± 0.34	13.87 ± 0.61	-	12.97 ±0.51
Methanol	17.33 ± 0.72	14.87 ± 0.85	8.03 ± 0.45	11.03 ± 0.45

* Tetracycline

Table 4.Antifungal activity of certain alcoholic leaf extracts of the species, *Senna tora*.

Plant extracts	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	<i>Aspergillus niger</i>	<i>A. flavus</i>	<i>A. baumannii</i>	<i>Fusarium xysporum</i>
Standard*	31.13 ± 0.59	33.27 ± 0.57	32.13 ± 0.14	32.13 ± 0.67
Petroleum ether	11.23 ± 0.49	-	-	11.17 ± 0.70
Ethyl acetate	12.73 ± 0.67	11.67 ± 0.32	12.77 ± 0.15	12.21 ± 0.71
Methanol	13.57 ± 0.70	14.11 ± 0.38	12.13 ± 0.80	10.87 ± 0.41

* Tetracycline

Table 5.Antifungal activity of certain alcoholic stem extracts of the species, *Senna tora*.

Plant extracts	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	<i>Aspergillus niger</i>	<i>A. flavus</i>	<i>A. baumannii</i>	<i>Fusarium xysporum</i>
Standard*	24.67 ± 0.41	23.17 ± 0.67	24.73 ± 0.15	30.73 ± 0.67
Petroleum ether	-	-	15.73 ± 0.28	-
Ethyl acetate	20.03 ± 0.52	14.67 ± 0.11	15.77 ± 0.71	11.67 ± 0.59
Methanol	10.73 ± 0.54	12.73 ± 0.70	14.67 ± 0.65	110.37 ± 0.38

* Tetracycline

Table 6.Antifungal activity of certain alcoholic root extracts of the species, *Senna tora*.

Plant extracts	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	<i>Aspergillus niger</i>	<i>A. flavus</i>	<i>A. baumannii</i>	<i>Fusarium xysporum</i>
Standard*	26.03 ± 0.21	30.63 ± 0.16	26.77 ± 0.65	27.67 ± 0.31
Petroleum	10.13 ± 0.54	13.23 ± 0.24	-	-
Ethyl acetate	20.53 ± 0.58	18.23 ± 0.32	19.27 ± 0.31	15.73 ± 0.70
Methanol	18.63 ± 0.41	16.63 ± 0.65	16.67 ± 0.61	14.17 ± 0.76

* Tetracycline

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