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ANTIMICROBIAL ACTIVITY OF THE MEDICINAL PLANT *SENNA OBTUSA* ROXB

M. KANNAN *, LIJA LJ, T. FRANCIS XAVIER, AND A. AUXILLIA

Department of Botany, St. Joseph's College, Tiruchirappalli–620 002, Tamil Nadu, India

*** Corresponding Author: E Mail: kannan.moorthy123@gmail.com**

ABSTRACT

The species, *Senna obtusa* Roxb. consists of small herbs found in tropical and subtropical regions and have wide application in herbal formulations. Leaf, stem and root used to cure various ailments in human beings. In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines researches in bioactive substances might lead to the discovery of new compounds that could be used to formulate new and most potent antimicrobial drugs to overcome the problem of resistant to the currently available antibiotics. The main objective of the present investigations is to analyze the fluorescence characters and evaluate the antimicrobial activity of crude extract of leaf, stem and root against selected gram positive and gram negative bacteria. The leaf and stem powder of the plants showed varying degree of antibacterial activity against all the tested bacteria.

Keywords: *Senna obtusa*, Bioactive Molecules, Antimicrobial Drugs, Crude Extract, Tested Bacteria

INTRODUCTION

Medicinal plants played an important role in the discovery of novel and useful drugs used in modern medicine. Today we have a number of drugs useful and life saving and also drugs which can provide immediate therapeutic benefit **[1]**. Over 2000 plant species are found to have medicinal value and are used for medicinal purpose in different forms. Many common plants seen in the Kitchen gardens or in the compound or in the forests are used by the tribal as medicines **[2]**. Our country is bestowed with rich and diverse resources of plant wealth including an enormously large number of medicinal plants. Plants are used medicinally in different countries and are

sources of many potent and powerful drugs. several herbs were known to possess medicinal values including antimicrobial properties **[3]**. Plant extracts of many higher plants have been reported to exhibit anti bacterial, anti fungal and insecticidal properties under laboratory trials.

The selection of crude plant extracts for screening programmes has the potential being more successful in its initial steps then the screening of pure compounds that the isolated from the natural products **[4]**. The world health organisation (WHO) has also reported that the large population of the world relies in the traditional system of medicine especially Indian and Chinese traditional medicinal plants. Due to this reasons, the trade value of Indian medicinal plants is expected to increase several times in the years to come **[5]**. The present study was designed to determine the role of aqueous and ethanol extract of *Senna obtusa* effectively inhibited the growth of both Gram positive and Gram negative bacteria.

MATERIALS AND METHODS

Plant Materials

Senna obtusa is an important medicinal plant belongs to the family of Caesalpiniaceae (alt. Fabaceae). It is an annual, erect, shrub like herb, pinnate leaves, yellow flowers, maxillary stalks and blooming in the month of august to September **[6]**. The roots extract of this plant contain tannins, flavonoids, alkaloids, betulinic acid, chrysophanol, physcion, stigmasterol **[7]**. Leaf extract of this plants were demonstrated a broad - spectrum of activities against both gram positive and gram negative bacteria & fungi that can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhoea, pneumonia, eye infections and mycotic infection **[8]**.

Plant Powder Preparation

The healthy plant samples (free from insect damaged, fungus - infected) were dried in the laboratory at room temperature for 5-8 days. Once completely dried, plants parts were ground to a fine powder using an electronic blender. Plants were stored in a closed container, at room temperature until required.

Preparation of Plant Extracts

Aqueous Extracts

50g of the dried powdered plant materials were extracted with 300ml of sterile distilled water (1:6 w/v). The aqueous extract was maintained in a soxhlet apparatus over 48hours. filtered and concentrated

Solvent Extracts

50g of the dried powdered plant materials (leaves and stems) were soaked separately with 300ml of each of the solvents *viz* ethanol, acetone, chloroform and petroleum

ether in a soxhlet apparatus for 48hrs. at 310º C until complete extraction of the materials. At the end of 48hrs each extract was filtered through Whatman No. 1 filter paper and filtrates were concentrated at room temperature in order to reduce the volume.

Test Microorganisms

9 Bacterial strains were used for the study, Gram $^{+}$ bacteria include, *Staphylococcus aureus, Bacillus cereus,* Gram^{-} bacteria include *Escherichia coli, Proteus vulgaris, Salmonella typhi, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumoniae* and *Enterobacter aerogens.* All the tested strains are reference strains and were collected from Doctor's Diagnostics. Thillainagar, Tiruchirappalli.

Antibacterial Assay

Disc Diffusion Assay

Sterile liquid Nutrient Agar Medium (pH 7.4 ±2) was poured (10- 15ml) into each sterile petriplates. After solidification 0.1 ml (100µl) of suspension containing 10^8 CFU/ml of each test bacteria were spread over Nutrient Agar plates. The sterile filter paper discs (6mm in diameter) were impregnated with 10µl of the 3mg/ ml extracts (30µg / disc) placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extract. Chloramphenicol (30µg/disc) were used has positive reference control to determine the sensitivity of plant extract on each bacterial species. the inoculated plates were incubated at 37ºC for 24hrs. Antibacterial activity was evaluated by measuring the zones of inhibition against the test organisms. Each assay was conducted in triplicate **[9]**.

RESULTS

The evaluation of the activity of the aqueous, ethanol, chloroform, petroleum ether, acetone extracts of leaf and stem of *Senna obtusa* against both Gram positive and Gram negative bacteria by using the disc diffusion method is given in **Tables 1 & 2**.

The *in vitro* results were observed in terms of inhibition zone around each disc caused by diffusion of antibacterial properties from the plant extract impregnated disc into the surrounding medium. Among various solvent extracts tested aqueous leaf extracts exhibited significant inhibition followed by ethanol, acetone and petroleum ether solvent extracts. The chloroform extracts did not show any antibacterial activity. In addition the inhibition zones formed by standard antibiotic disc (Chloramphenicol 30 mg/disc) and those filter paper discs injected with ethanol, acetone and petroleum ether are also listed in the **Tables 1 & 2**.

The diameter of inhibition zones for each of the samples were compared with standard

antibiotics. It was noted that the inhibition zones of the samples to be either less than or greater than or equal to the inhibition zones of standard antibiotics.

The leaf extracts exhibited high degree of inhibition than the other parts used. The diameter of inhibition zones were noted in the leaf extracts, the aqueous extract showed greater antibacterial activity against the test bacteria. The zone of inhibition was higher in the case of *Klebsiella pneumoniae* (10mm) and *Enterobacter aerogens* (10mm).

Moderate inhibition was observed in *Proteus vulgaris* (7.0mm) and *Serratia marcescens* (7.0mm), *Staphylococcous aureus* (6.0mm), *Bacillus cereus* (5.0mm). the results of the antibacterial screening of other solvent extracts of leaf and stem are also shown in **Tables 1 & 2.**

Chloroform extract showed complete absence of inhibition zones and in some occasion petroleum ether and acetone extracts exhibited complete absence of inhibition against *Bacillus cereus, Proteus vulgaris, Enterobacter aerogens*. The aqueous and ethanol extract of *Senna obtusa* effectively inhibited the growth of both gram positive and gram negative bacteria.

In conclusion, it is suggested that the plant may be recommanded as useful sources to prepare natural bioactive products from which we can develop new antibacterial drugs which will be cost effective because the plant is freshly available. In the search new pharmaceuticals, screening of such various natural organic compounds and identification of active agents must be considered as a fruitful approach.

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Table 1: Antibacterial Activity of Leaf Extracts of *Senna obtusa* on Pathogenic Bacteria

S. No.	Test Bacteria	Inhibition zone diameter in cm [mean ± SD]					
		Aqueous*	Ethanol*	Petroleum ether*	Acetone*	Negative control ethanol leaf extract	Positive Control Chloramphenicol
1.	*Staphylococcous aureus*	6.0±0.14	1.0±0.00	7.0±0.4	7.0±0.4	2.0±0.00	a
2.	*Bacillus cereus*	0.5±0.5	2.0±0.47	a	a	2.0±0.47	1.5± 0.00
3.	*Escherichia coli*	6.0±0.10	2.0±0.00	9.0±0.6	9.0±0.6	2.0±0.00	3.1 ± 0.00
4.	*Proteus*	7.0±0.25	2.0±0.00	a	8.0±0.5	2.0±0.00	3.1±0.00
5.	*Salmonella typhi*	5.0±0.00	7.0±0.40	a	a	1.0±0.00	2.4±0.0
6.	*Pseudomonas aeruginosa*	5.0±0.50	4.0±0.20	a	a	2.0±0.00	A
7.	*Serratia marcescens*	7.0±0.50	5.0±0.30	a	a	1.0±0.00	a
8.	*Klebsiella pneumoniae*	10.0±0.80	1.0±0.04	1.2±0.4	8.0±0.5	2.0±0.04	2.4 ±0.00
9.	*Enterobacter aerogens*	10.0±7.0	1.0±0.00	a	a	2.0±0.00	2.6 ±0.00

NOTE: *: Subtracted Value From Negative Control; a: No Inhibition

Table 2: Antibacterial Activity of Stem Extracts of *Senna obtusa* on Pathogenic Bacteria

S. No.	Test Bacteria	Inhibition zone diameter in cm [mean ± SD]					
		Aqueous*	Ethanol*	Petroleum ether*	Acetone*	Negative control ethanol leaf extract	Positive Control Chloramphenicol
1.	*Staphylococcous aureus*	a	a	7.0±0.4	a	2.0±0.00	a
2.	*Bacillus cereus*	a	a	a	a	2.0±0.47	1.5±0.00
3.	*Escherichia coli*	a	a	9.0±0.6	a	2.0±0.00	3.1±0.00
4.	*Proteus vulgaris*	a	a	a	a	2.0±0.00	3.1±0.00
5.	*Salmonella typhi*	9.0±0.7	8.0±0.4	8.0±0.4	a	1.0±0.00	2.4±0.0
6.	*Pseudomonas aeruginosa*	a	5.0±0.2	5.0±0.2	a	2.0±0.00	a
7.	*Serratia marcescens*	a	6.0±0.3	6.0±0.3	a	1.0±0.00	a
8.	*Klebsiella pneumoniae*	9.0±0.7	a	9.0±0.3	9.0±0.7	2.0±0.04	2.4 ±0.00
9.	*Enterobacter aerogens*	a	a	a	a	2.0±0.00	2.6±0.00

NOTE: * : Subtracted Value From Negative Control; a : No Inhibition