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ANTIMICROBIAL ACTIVITY OF BARK EXTRACTS OF *SESBANIA SESBAN* (L) MERR.

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ABSTRACT

Sesbania Sesban is a short lived shrub upto 8 m tall, which belongs to the family *Fabaceae*. The bark of *sesbania sesban* has been traditionally used as an astringent, in diarrhea, ulcers, bronchitis, tumours, cirrhosis of liver, hypertension & spleen enlargement. In this present study an antimicrobial activity of the petroleum ether, chloroform and methanol extracts of bark of *Sesbania sesban* against five Gram-positive bacteria by disc diffusion was performed. The zone of inhibition was observed with almost all bacteria and some exceptions. In conclusion, the bark extract of *Sesbania sesban* possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases.

Introduction:

Nature is a unique source of structures of high phytochemical diversity, many of them possessing interesting biological activities and medicinal properties. The phytochemicals present in plants are responsible for preventing disease and promoting health have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Secondary metabolites or phytochemicals such as phenols, flavonoids, alkaloids, terpenoids, and essential oils are proved to be responsible for the antimicrobial activity of plants. These secondary metabolites are not essential for the plant itself; however they play an important role in plant's defense system and give protection against pathogens and herbivores. *Sesbania sesban* (L) Merr. is an ethanobotanically important plant which is widely used in traditional medicine system. The phytochemical analysis of the petroleum ether, chloroform and methanol extracts of bark of *Sesbania sesban* revealed the presence of flavonoids, alkaloids, steroids, phenols, carbohydrates, other chemical compounds such as, tannins, saponins, terpenoids, amino acids and phytosterols, which might be responsible for their therapeutic effects. Various natural products are potent antimicrobial agents without side-effects. Such studies have included identification and isolation of the chemical components, establishment of their biological potency both by *in vitro* and *in vivo* studies in experimental animals and through epidemiological and clinical-case control studies in man. In the present work, we have investigated the antimicrobial activity of different extracts of bark of *Sesbania sesban* and also identified the extracts which possess most prominent antimicrobial activity.

Material and methods:**Plant material:**

Bark of plant *Sesbania sesban* (L) Merr. was collected from Hatti village in Dhule district of Maharashtra and later on authenticated by Mrs. J. Jayanthi, Head of the department, Botanical Survey of India, Pune, where herbarium voucher specimen No. (RKC-1) has been deposited.

Extraction and isolation:

The bark was dried under shade with ample aeration. After complete drying, the plant material was grinded into a coarse powder and extracted with different solvents successively. The coarse powder was first extracted with petroleum ether (60-80°C) using Soxhlet apparatus. After complete extraction the remaining marc was dried to remove the solvent. The marc was then

successively extracted with chloroform and methanol. Extracts were concentrated by vacuum distillation and then dried in open air to produce respective extracts. The dried extracts were refrigerated until use.

Anti-microbial testing:

E. coli, *S. aureus*, *Pseudomonas aeruginosa*, *Aspergillus*, *Candida* were used in antimicrobial tests. Antimicrobial activity was investigated using the method described in the Indian Pharmacopeia.

a) Cup plate and agar diffusion method was used to determine the zone of inhibition of three extracts of bark of *Sesbania sesban*. In brief, molten nutrient agar cooled to 45⁰c was inoculated with different test organisms and then petroleum ether, chloroform and methanolic extracts of bark of *Sesbania sesban* having concentrations 1mg/ml were placed in the cup, following incubation for 24 hours. The plates were observed for the zone of inhibition around the cup. The experiment was performed thrice.

b) Sterile nutrient broth: (double strength) was used to determine the minimum inhibitory concentration (MIC) of the extracts. Stock solutions of petroleum ether, chloroform and methanolic extracts of bark of *Sesbania sesban* having 2 mg/ml conc were used. Sterile dilutions in nutrient broth were done by using the stock solution to get lowest conc. of 0.0019 mg/ml. In even set of tubes two loops full of suspension of the micro-organism stated above were inoculated. All culture tubes were incubated at 37⁰c for 24 hrs. Following incubation, the tubes were examined for presence of microbial growth. The lowest conc inhibiting microbial growth was considered to be MIC. The experiment was repeated thrice.

The following Antimicrobial tests were conducted in Department of Microbiology by Kirby Bauer method and the results of the same are given in the table below.

Name of Sample: - Sample 3 (petroleum ether containing)

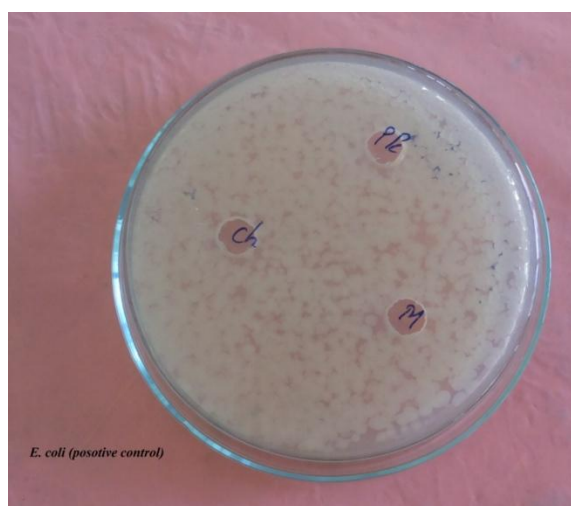
Name of Bacteria/Fungi	Positive control	Zone of inhibition
<i>E.coli</i>	Nil	No Zone of inhibition
<i>S.aureus</i>	Nil	No zone of inhibition
<i>Pseudomonas aeruginosa.</i>	Nil	No Zone of inhibition
<i>Aspergillus</i>	Nil	No zone of inhibition
<i>Candida</i>	Nil	No Zone of inhibition

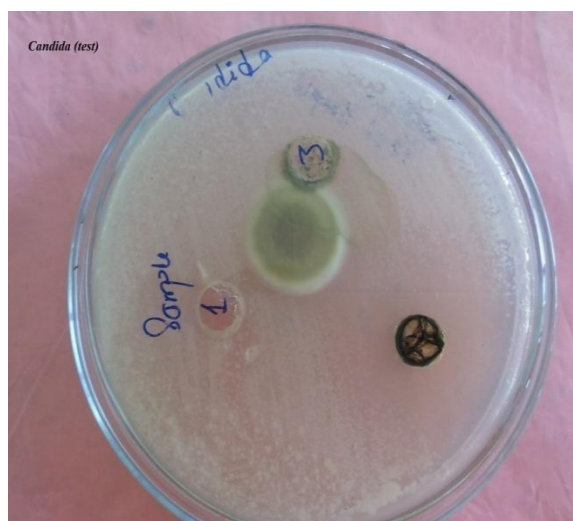
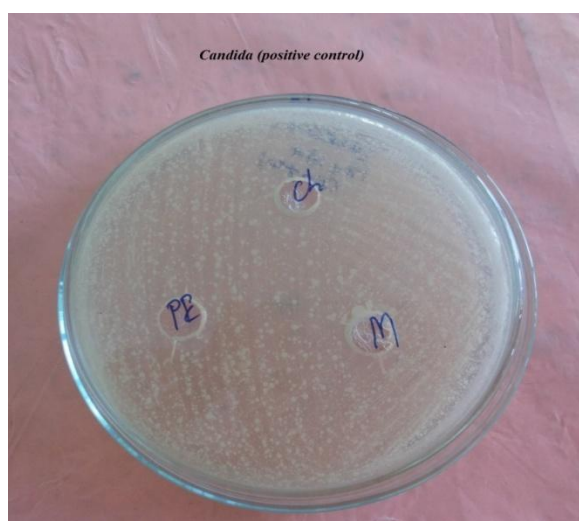
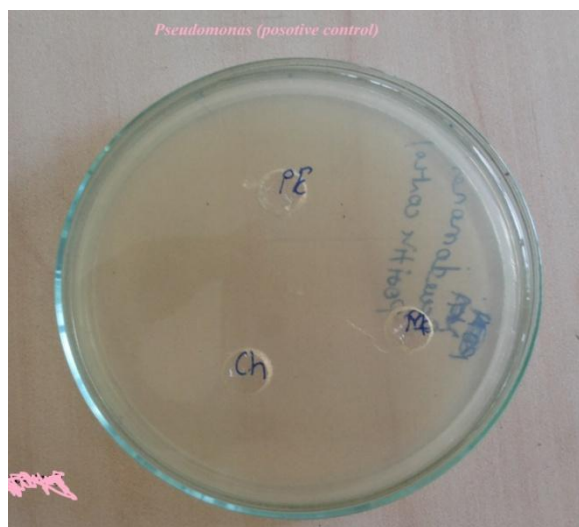
Name of Sample: - Sample 2 (Chloroform containing)

Name of Bacteria/Fungi	Positive control	Zone of inhibition
<i>E.coli</i>	Nil	24mm
<i>S.aureus</i>	Nil	No zone of inhibition
<i>Pseudomonas aeruginosa.</i>	Nil	13mm
<i>Aspergillus</i>	Nil	No zone of inhibition
<i>Candida</i>	Nil	29mm

Name of Sample: - Sample 1 (Methanol containing)

Name of Bacteria/Fungi	Positive control	Zone of inhibition
<i>E.coli</i>	Nil	No zone of inhibition
<i>S.aureus</i>	Nil	No zone of inhibition
<i>Pseudomonas aeruginosa.</i>	Nil	No zone of inhibition
<i>Aspergillus</i>	Nil	No zone of inhibition
<i>Candida</i>	Nil	No zone of inhibition





Results and Discussion:

The Chloroform extract as well as methanolic extract exhibited prominent antimicrobial activity against *E.coli*, *Pseudomonas aeruginosa*, *Candida* microorganisms used in the study. From the zone of inhibition produced by the extract, it was observed that, the chloroform extract showed prominent anti-microbial activity against *Candida*, *E.coli* and *Pseudomonas aeruginosa* as compared to other tested microorganisms. None of the negative control exhibited anti-microbial activity. Thus the solvent used for solubilization of drug had no anti-microbial activity. Above table shows the average value of zone of inhibition of various extracts of *Sesbania sesban* bark used in the study. The zone of inhibition of chloroform extract against *Candida* is 29mm, for *E.coli* is 24mm and for *Pseudomonas aeruginosa* is 13mm.

Thus the chloroform extract exhibited antimicrobial activity, and it is more potent as compared to methanolic and petroleum ether extracts.

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