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Anti-bacterial and anti-fungal evaluation of bark extracts of *Sesbania grandiflora* L.

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Abstract---The aim of the present study is to evaluate the antibacterial and antifungal activity of bark extracts of *Sesbania grandiflora* L. The antibacterial screening was done by Paper disc method and antifungal screening by Disc Diffusion method using pet ether, chloroform, aqueous and methanolic extracts. The results showed strong potential of both the activity of methanolic extracts as compared with that of the other extract.

Keywords---*Sesbania grandiflora*, Anti-bacterial activity, Antifungal activity, Paper disc method, Disc Diffusion method, streptomycin, amphoterecin etc.

Introduction

Antioxidants are substances used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals. Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals [Aurelia et. al. 2011]. Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxy nitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases [Roy et.al.2014]. *Sesbania grandiflora* which belongs to family Fabaceae commonly known as 'sesbania', is widely used as Indian folk medicine. *S. grandiflora* has the common names of Agati, Corkwood Tree and West Indian Pea, The hummingbird tree (or scarlet wisteria). In India, it is known as vaka or basna.

Traditionally *Sesbania grandiflora* is used alone or with other medicinal plants to treat a variety of ailments. It is a small tree believed to have originated either in India or Southeast Asia and grows primarily in hot and humid tropical areas in the world. A native to Asian countries such as India, Malasia, Indonesia and the Philippines where it is commonly seen growing on the dikes between rice paddies, along roadsides and in backyards vegetable gardens. The whole plant contains amino acids, phenolic compounds, flavonoids and a saponin yielding oleanolic acid, galactose, rhamnose and glucuronic acid and it also contains flavonol glycoside, kaempferol. The root-bark of the red-flowered variety is useful in vitiated condition of vata and arthralgia. The bark is astringent, cooling, bitter, tonic, anthelmintic and febrifuge. The pounded bark is externally applied to cure scabies. The juice of the bark is good for dyspepsia, diarrhea and gastralgia. The leaves are acrid, bitter, sweet, cooling, aperient, tonic and diuretic and contain a non-poisonous saponin like substance. The flowers are cooling, bitter, astringent, acrid and antipyretic. The fruits are sweet, bitter, laxative and alexiteric and are useful in flatulent-colic, astringent, cooling, bitter, tonic, anthelmintic, febrifuge, cure scabies, dyspepsia, diarrhea and gastralgia, astringent, antipyretic [(Kirthikar and Basu,1998), (Chatterjee,1992), (Rastogi,1960)]. Based on the above medicinal properties of *Sesbania grandiflora*, in this study, we investigated the anti-bacterial activity and antifungal activity of pet ether, chloroform, methanolic and aqueous extracts of plant bark.

Materials and Methods

Plant Material

The plant material of *Sesbania grandiflora*. (*Fabaceae*) bark was collected from local area of Dhule district, Maharashtra, India. The plant material was clean and dried. Also was identified and authenticated from Department of Botany, S.S.V.P.S's L. K. Dr. P. R. Ghogarey Science College, Dhule (M.S.) by Voucher Specimen No.110.

Preparation of the Extract

Dried bark material were mechanically reduced to a coarse powder and then sieved and stored in an air tight container at room temperature. The extraction method was based on the presence of active constituents in the drug, using various solvents ranging from non-polar to polar. Dried powder was extracted sequentially with pet ether, chloroform, methanol and distilled water by using soxhlation method. The extracts were concentrated to dryness by distilling the solvent at low temperature using rotary evaporator. The extract was stored in airtight container.

Antibacterial activity

Paper disc method

[(Anita and Subramaniam, 2012), (Manore, 2012), (Valgas, 2007), (Bose and Bose,2008)]

Test organism: *Escheria coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus substilis*.

Nutrient Medium: Culture of the test organism was maintained on nutrient agar slants and was subcultured in petri dishes to testing.

Composition of Nutrient agar medium:

Peptic digest animal tissue – 1gm, Sodium chloride – 1gm, Beef extract – 0.3gm, Yeast extract – 0.3gm, Agar – 6gm, P.H – 7.4, Distilled water 200ml.

Sterilization of Media: The sterilization of media was done by autoclaving at 15lb/sq. inch pressure for 30 min.

Sterilization of other materials; The sterilization of cultures tubes, pipettes, and other materials was done by hot air oven at 160°C for 60 min.

Preparation of standard drug: Streptomycin (1mg/ml) in DMSO.

Preparation of extract solution: Petroleum ether, Chloroform, Methanolic and Aqueous extracts of the plants *Sesbania grandiflora* were separately dissolved in dimethyl sulfoxide (DMSO) to get 10 mg/ml solutions.

Procedure

The in vitro antibacterial study of the *Sesbania grandiflora* bark extract was carried out by the paper disc method. The nutrient agar plates were prepared by pouring 20ml of molten media in to sterile petri plates under aseptic condition. The plates were allowed to solidify for 5 minutes and 0.5ml of test culture was inoculated and uniformly spread over the agar surface with the help of a sterile L-shaped bent glass rod. After inoculation, wells were scooped out with 6mm sterile cork borer and the lids of the dishes were replaced. To each well different extract solution were added separately and controlled were same maintained. The treated plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the discs were measured and diameter was calculated in millimeter.

Antifungal Activity:

[Punnagai et.al.2016]

Disc Diffusion method

Test organism: *Candida albicans*

Sterilization of Media: - The sterilization of media was done by autoclaving at 15lb/sq. inch pressure for 30 min.

Sterilization of other materials;- The sterilization of cultures tubes, pipettes, and other materials was done by hot air oven at 160°C for 60 min.

Preparation of standard drug:-Amphotericin B (1mg/ml) in DMSO.

Preparation of extract solution:-Petroleum ether, Chloroform, Methanolic and Aqueous extracts of the plants *Sesbania grandiflora* were separately dissolved in dimethyl sulfoxide (DMSO) to get 10 mg/ml solutions.

Procedure

The Assay was performed by agar disc diffusion method. Active cultures for the experiment were prepared by transferring a loop of full of culture from the stock cultures into the test tubes containing Sabouraud Dextrose broth , that were incubated at 48hrs at 37°C. Antifungal activity of the plant extracts was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured in to the petriplate. After the

medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. The disc were placed in SDA plates and add 20 μ l of sample (Concentration: 1000 μ g, 750 μ g and 500 μ g) were placed in the disc. The plates were incubated at 37°C for 24 hrs. The antifungal activity of the plant extract was determined by measuring the diameter of the inhibitory zones using a transparent ruler and compared with those produced by the antibiotic Amphotericin B (20 μ g/disc) Amphotericin B is used as a positive control and the diameter of zones of inhibition less than 5 mm were considered as insignificant.

Results

Antibacterial Activity

Table 3.1
Result of Antibacterial activity of *Sesbania grandiflora* plant

Extract	Zone of inhibition diameter in mm.			
	E Coli	Pseudo aerugi	Staph. aureus	Bacillus substilis
Pet. ether extract of <i>S. grandiflora</i>	10	15	14	—
Choloroform extract of <i>S. grandiflora</i>	10	10	10	—
Methanol extract of <i>S. grandiflora</i>	10	10	16	—
Control	10	—	11	—
Aqueous extract of <i>Sesbania grandiflora</i>	—	—	10	—
Standard streptomycine	29	26	25	22

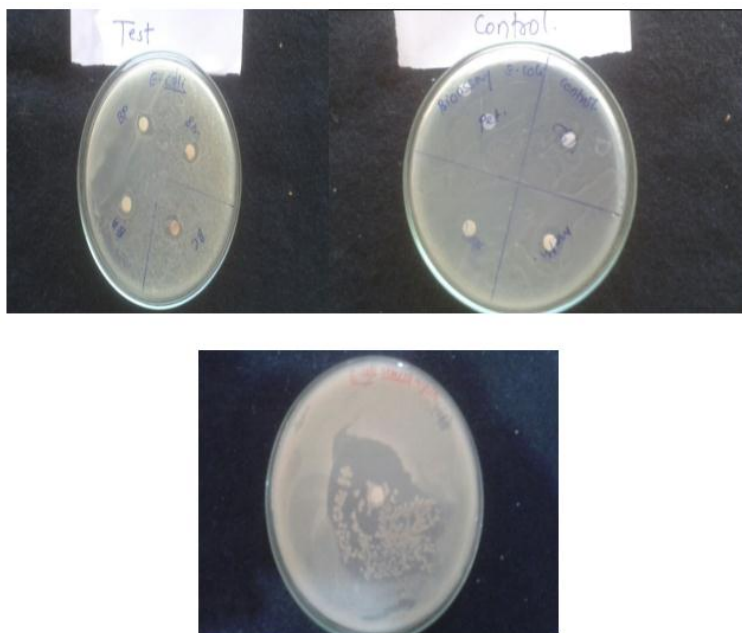


Figure 3.1 Antibacterial activity against *E.coli*

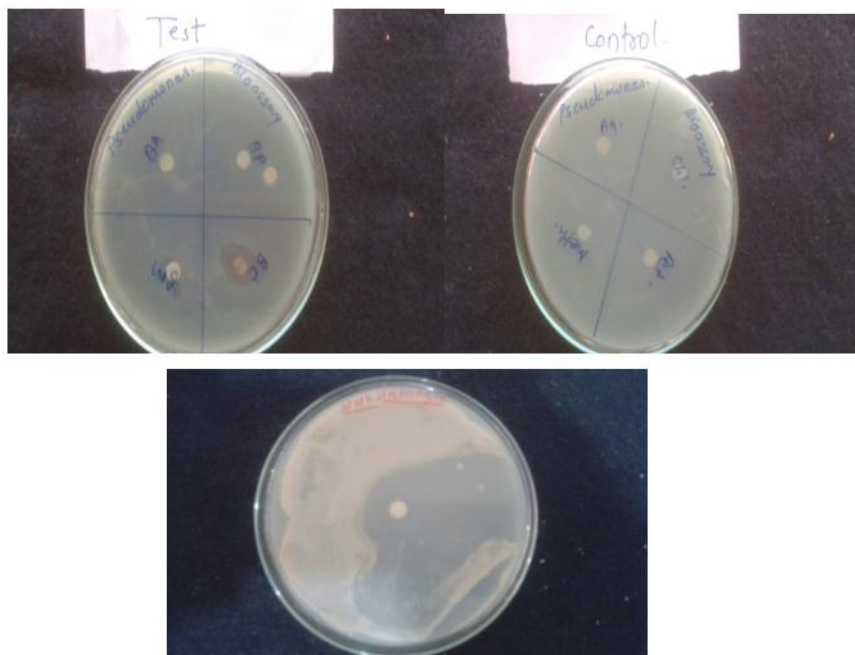


Figure 3.2 Antibacterial activity against *pseudomonas aeruginosa*

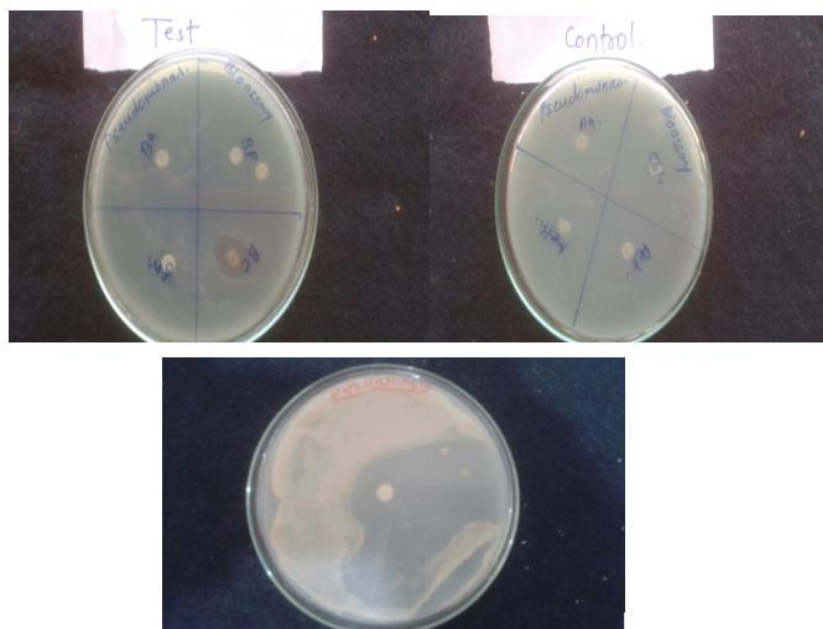


Figure 3.3 Antibacterial activity against *staphylococcus aureus*

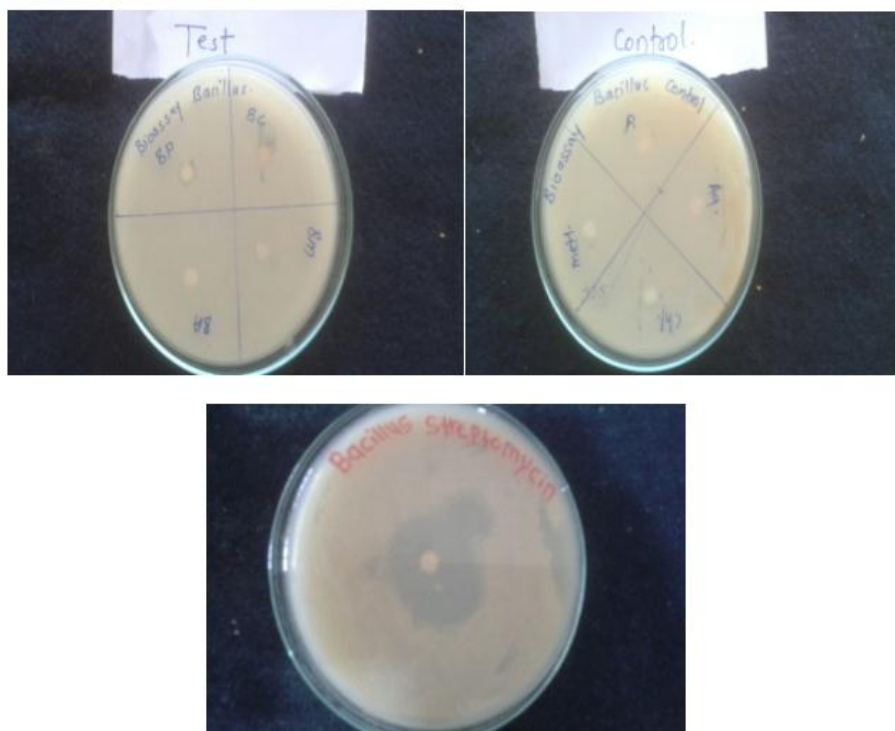


Figure 3.4 Antibacterial activity against *Bacillus Subtilis*

Where

A - Petroleum ether extract of *S. grandiflora*, B- Choloroform extract of *S. grandiflora*.
C - Methanol extract of *S. grandiflora*. D- Aqueous extract of *S. grandiflora*

Antifungal Activity

Table 3.2
Result of Antifungal activity of *Sesbania grandiflora* plant

Extract	Zone of inhibition diameter in mm.
	<i>Candida albicans</i>
Pet. ether extract of <i>S. grandiflora</i>	08
Choloroform extract of <i>S. grandiflora</i>	05
Methanol extract of <i>Sesbania grandiflora</i>	12
Aqueous extract of <i>Sesbania grandiflora</i>	09
Standard Amphotericin B	16

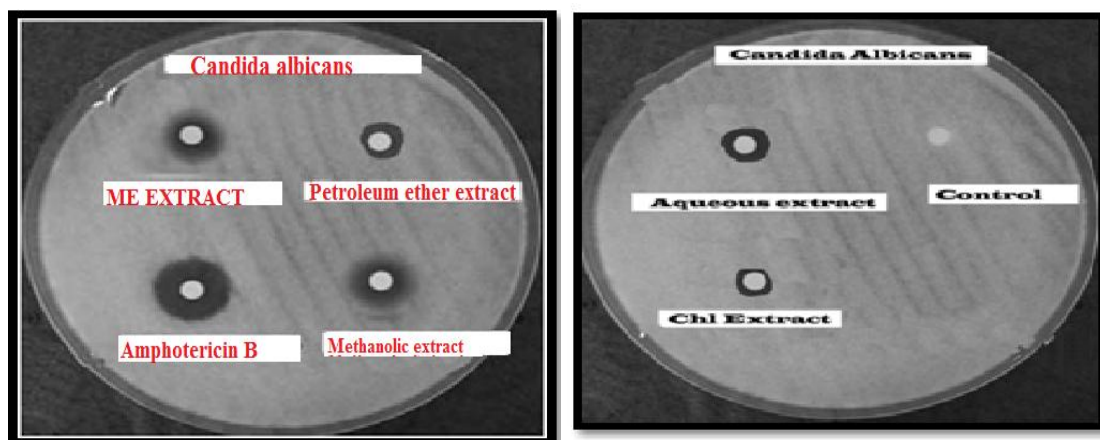


Figure 3.5 Antifungal activity against *Candida albicans*

Discussion and Conclusion

The antibacterial activity of various extracts of plants *Sesbania grandiflora* were studied by the disc diffusion method by using standard streptomycin. The Methanolic extract of *Sesbania grandiflora* shows better antibacterial activity as compared to petroleum ether, chloroform and aqueous extracts. The Streptomycin is used as a standard antibiotic was showed higher activity against all microorganisms when compared with all extracts. The results were shown in Table no. 3.1 & Fig no. 3.1-3.4.

The antifungal activity of various extracts of plants *Sesbania graniflora* were studied by the disc diffusion method. The methanolic extracts of *Sesbania grandiflora* shows antifungal activity. The methanolic extract of *Sesbania grandiflora* shows better antifungal activity as compared to aqueous, chloroform and petroleum ether extracts. The Amphotericin-B were used as standard. The results were shown in Table no. 3.2 & Fig. no. 3.5.

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