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## Antimicrobial activity of natural dyes obtained from *Alstonia Scholaris* barks

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### Abstract

In the present study to evaluate the antimicrobial activity of *Alstonia Scholaris* barks. Many of the plant materials, from which natural dyes are obtained, found to have some medicinal values. During the present study, dying materials were prepared from barks of *Alstonia Scholaris*. The well diffusion method was adopted to examine antimicrobial activity of dying materials against test organisms. The result showed that the dying materials of *Alstonia Scholaris* was most active for antibacterial (10 mm) and antifungal (11 mm). Results of the present study suggest that the dying material of *Alstonia Scholaris* has significant antibacterial activity against pathogenic bacteria and fungus.

**Keywords:** *Alstonia Scholaris*, Antimicrobial activity, Well diffusion method, Natural dyes

### 1. Introduction

The plant of *Alstonia scholaris* (L.) R. Br. belongs to family Apocynaceae and is also known as Devil's tree or Dita Bark tree. The plant grows throughout India, in deciduous and evergreen forests and also in plains [1]. *Alstonia scholaris* is an antimalarial drug used in the marketed Ayurveda preparation Ayush-64, NRDC, India. The milky juice of the plant is applied on wounds, ulcers and rheumatic pains. Tincture of the bark and juice of the leaves act as powerful galactagogue in certain cases. The drug is also used in case of snake bite [2, 3]. Different parts of this plant are used in traditional medicines. The bark is used for its tonic bitter and astringent properties; it is particularly useful for chronic diarrhoea and dysentery, indigestion and typhoid. It acts as galactagogue, stomachic, laxative and liver tonic. Jahan *et al.* demonstrates the chemo preventive potential of *Alstonia scholaris* bark extract in DMBA-induced skin tumorigenesis in Swiss albino mice [4].

Hadi and Bremner tested *A. scholaris* for antimalarial properties. *A. scholaris* showed significant luteolytic and anti-implantational effect in rats [5]. The new indole alkaloid, alstonamine and a sibirikine type indole alkaloid, rhazimanine, have been isolated from the leaves of *A. scholaris* [6]. Hadi and Bremner (2001) tested *A. scholaris* for antimalarial properties of alkaloids obtained from young plants leaves. Patrick *et al.* (2005) isolated different types of alkaloids from the leaves of the Philippine medicinal plant, *A. scholaris*. The present research is focused on antibacterial activity of *A. scholaris* [7, 8]. Qualitative tests of *Alstonia scholaris* methanol extract done by Khyade and Vaikos suggested that plant is rich in iridoids, alkaloids, coumarins, flavonoids, phlobatannin, reducing sugars, simple phenolics, saponins and tannins [9]. Banerji and Banerji reported that a large number of alkaloids, steroids and triterpenoids are present in *A. scholaris* [10].



Photo 1: *Alstonia Scholaris*

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Photo 2: Bark pieces of *Alstonia Scholaris*

## 2. Material and Methods

### 2.1 Collection of bark materials

The barks of *Alstonia Scholaris* was collected from Hogenakkal Cauvery River, Dharmapuri District, Tamil Nadu. The botanical identity of the plant of was confirmed by Dr. S. John Britto, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli.

### 2.2 Preparation of Dying Material

The small pieces of *Alstonia Scholaris* bark (5 g) was extracted with 40% ethanol at room temperature for one day. The extract was filtered and concentrated under reduced pressure in a rotary evaporator and extracted dying material was boiled with 68 °C than cooled. The dying material were subjected to antimicrobial activity.

## 3. Experimental Work

### 3.1 *In-vitro* antimicrobial activity (Well diffusion method)

The dying material were prepared 100 ppm concentration were used for antimicrobial activity.

### 3.2 Test microorganisms

Pure cultures of *Bacillus Pumilus*, *Bacillus Cereus*, *Escherichia coli*, *Salmonella SPS* (Gram positive bacteria), *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Gram negative bacteria) specie of bacteria's and *Candida albicans*, *Aspergillus flavus* specie of fungi's were procured from Rontgen Laboratory, Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur.

### 3.3 Preparation of 24 hours' pure culture

A loop full of each of the microorganisms was suspended in about 10 mL of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37 °C for 24 hours except for fungal which was incubated at 25 °C for 24-48 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8 °C until use.

### 3.4 Preparation of dying material solutions for the experiment

The dying material was dissolved in sterile distilled to prepare appropriate dilution to get required concentration. Control used as respective solvent (Aqueous). They were kept under refrigerated condition unless they were used for the experiment. Standard solution as Chloramphenicol for

bacteria and fluconazole (25 mg/mL distilled water - 30 µL) for fungi used to compare the test solution. They were kept under refrigerated condition unless they were used for the experiment.

The plates were incubated at 5 °C for 1 hour to permit good diffusion and then transferred to incubator at 37 °C for 24 hours. After completion of 24 hours, the plates were inverted and placed in an incubator set to respective temperature for 24 hours.

### 3.5 Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.* 2007) [11] using plant extracts. Petri plates were prepared by pouring 30 mL of NA/PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 minutes.

The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Bacillus Pumilus*, *Bacillus Cereus*, *Escherichia coli*, (Gram positive bacteria), *Salmonella SPS*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Gram negative bacteria) specie of bacteria were spread on Nutrient agar plates for bacteria and *Candida albicans*, *Aspergillus flavus* specie of fungus were spread on potato dextrose agar for fungus strains. The plates were incubated at 37 °C for 24 hours for the bacteria and 48 hours for fungus at room temperature (30±1) for 24-48 hour for yeasts strains. Each sample was tested in triplicate.

Table 1: Anti-bacterial activity of dying material of *Alstonia Scholaris* bark

S. No	Name of Organism	Diameter in mm
<b>Gram positive bacteria</b>		
1	<i>Bacillus Pumilus</i>	9 mm
2	<i>Bacillus Cereus</i>	7 mm
3	<i>Escherichia coli</i>	10 mm
<b>Gram negative bacteria</b>		
4	<i>Salmonella SPS</i>	8 mm
5	<i>Pseudomonas aeruginosa</i>	9 mm
6	<i>Staphylococcus aureus</i>	Nil

Table 2: Anti-fungal activity of dying material of *Alstonia Scholaris* bark

S. No	Name of Organism	Diameter in mm
1	<i>Aspergillus flavus</i>	11 mm
2	<i>Candida Albicans</i>	6 mm

## 4. Results and Discussion

Result obtained in the present study the antimicrobial activity of the *Alstonia Scholaris* bark shown in table 1 & 2. The result shows dying material of *Alstonia Scholaris* was effective against both antibacterial and anti-fungal activities. For antibacterial activity was recorded as the *Escherichia coli* at 10 mm, *Bacillus Pumilus* at 9 mm, *Bacillus Cereus* 7 mm, *Salmonella SPS* 8 mm and *Pseudomonas aeruginosa* 9 mm when compared with chloramphenicol as standard. For anti-fungal activity of *Aspergillus flavus* 11 mm and *Candida Albicans* 6 mm was observed when compared with nystatin as standard. The antimicrobial activity of the *Alstonia Scholaris* bark was effective against both antibacterial and anti-fungal activities.

## 5. Conclusion

In the present study dying material of *Alstonia Scholaris* bark indicate that maximum activity of both gram positive, negative bacteria and fungi's. Hence the dying material of *Alstonia Scholaris* bark was worthy for further investigation as used as some natural drugs developments.

## 6. Acknowledgement

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