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# Antibacterial Spectrum and Activity Guided Evaluation of Bark of *Sonneratia Alba*

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**Abstract:** ***The present study has been undertaken to investigate bioactive compounds from bark of Sonneratia alba. Extracts were made in acetone, ethyl acetate and water by using powder of bark of S. alba. Bioassay of primary screening and MIC were done by disc diffusion method using various gram negative and gram positive bacteria. Maximum inhibition was shown against Klebsiella pneumoneae by acetone and aqueous extract and against Corynebacterium diphtheriae by aqueous extracts. MIC of all potent extracts displayed promising inhibitory activity against susceptible microorganisms. Activity Guided Fractionation of highly potent extracts indicated active principles mainly dissolved in polar solvents.***

**Keywords:** Bioactive compounds, *S. alba*, Bioassay, MIC and activity guided fractionation

## 1. Introduction

Since ancient times plants have played an important role in the discovery of new therapeutic agents. Much of the scientific efforts made in the past few decades with medicinal plants have focused on documenting the uses of traditional medicine, effectiveness of particular remedies characterizing medicinal plants to their compound and testing plant compounds *in vitro*. **[1]**, **[2].**

A special feature of higher plants is their capacity to produce a large number of secondary metabolites which are produced for self defense. **[3]**. Human beings still rely on plants to combat bacterial and fungal infections. Microorganisms have developed a resistance to many antibiotics and as a result, immense clinical problems in the treatment of infectious diseases have been created. **[4].**

## 2. Literature Survey

Mangroves are 'coastal woodlands' or marine tidal forests comprising of trees, shrubs, palms, epipyhytes, ground ferns and grasses. They occupy large tracts along sheltered coasts, estuaries and in deltas. They are specially adapted to withstand salinity, wave action and can grow in poor soil. Mangroves occur approximately in 112 countries. **[5]**. The Sundarbans are the largest mangrove forest in the world, both in size as well as biodiversity. Mangroves are important source of tannins used for dying, leather production and oil drilling. They provide food, timber, variety of traditional products and folklore medicine. **[6]** and **[7]**. Mangroves are biochemically unique producing a wide array of novel natural product. **[8]**. Several biological activities are attributed to different mangroves. **[9]**, **[6]** and **[7]**

Various phytochemicals are well known for their antimicrobial properties and can be of great significance in therapeutic treatments. Increasing resistance to the antimicrobial agents is very common in patients with various infections. **[10]**.

*S. alba* consists secondary metabolites like steroids, polyphenols, glycosides and condensed as well as hydrostable tannins. The pneumatophores of *S. alba* and *S. caseolaris* are used in making corks, fishing floats and heels of shoes. **[11]**. The leaves, trunk and bark have been reported for its antoxidant properties. **[12]**. Folklore medicinal uses of *S. alba* include treatment of various skin ailments. **[13]**.

Activity guided fractionation of various mangroves were studied. **[14]**, **[15]**. Characterization of active principles of *S. apetela* was studied by various researchers. **[16]**, **[17]** and **[18]**. *S. apetela*, *R. mucrunata* and *B. cylindrica* were found to be highly potent against various pathogens. Antimicrobial activities of various parts of *S. alba* made in organic solvents were also investigated. **[19]** and **[20]**.

## 3. Problem Definition

There is an urgent need to explore and discover new antimicrobials with diverse chemical and novel mechanical action for new and re-emerging infectious diseases. In recent years secondary plant metabolites, previously with unknown pharmacological activity have been extensively investigated as a source of medicinal agents. In the context of this a study was undertaken to evaluate the activity of bark of *S. alba* against several gram positive and gram negative bacteria *in vitro.*

## 4. Materials and Methods

### Plant Material

Mangrove species *S.alba* was collected from Mumbra estuaries, District Thane, Maharashtra, India. The bark was washed with tap water and dried in shade, finely powdered and stored in airtight bottle.

### Preparation of Extracts

1 gram of air dried powder of bark was immersed in 20 ml of organic solvents acetone, ethyl acetate, and distilled water separately to make crude extracts. It was incubated at room temperature for 48 hours at 150 rpm on an orbital shaker. The suspensions were filtered, made 20 ml and stored at 4°C for antimicrobial activity.

**Microorganisms**
Six gram negative bacteria *Escherichia coli (E.coli), Klebsiella pneumoneae (K.p), Salmonella typhii (S.t), Salmonella paratyphii A (Sp.A), Salmonella paratyphii B (Sp.B)* and *Pseudomonas aeruginosa (Ps.aeru)*, four gram positive bacteria *Staphylococcus aureus (S.a), Bacillus subtilis (B.subt), Streptococcus pyogens (S.pyo)* and *Corynebacterium diphtheriae (C.d)* were used for bioassay. All the strains were collected from Haffkine Institute, Parel, Mumbai and sub cultured at regular intervals. These microbes were maintained on nutrient agar slant and stored at 4°C.

**Media used**
Nutrient agar and nutrient broth (Hi Media).

**Preparation of Inoculums**
Active cultures for experiments were prepared by transferring a loopful of culture to nutrient agar slant (Hi Media), incubated at 37°C for 24 hours for bacterial proliferation.

**Bioassay**
Paper disc method **[21]** was employed for testing antibacterial activity. Sterile sugar tubes with 20-25 ml sterile nutrient agar and18-24 hours old 0.4 ml of culture made in nutrient broth of each pathogen, mixed and poured on each sterile plate. The plates were kept aside for agar solidification. Paper disc (5 mm in diameter) soaked into plant extract were placed onto the plates and the plates were incubated at 37°C for 24 hours **[22]** to allow the bacterial growth. The antibacterial activity of the bark extract was determined by comparing the diameter of the zone of inhibition with that of respective controls (acetone, ethyl acetate, and distilled water). For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The experiment was conducted in triplicate and the mean values are represented.

**Minimum Inhibitory Concentration (MIC)**
In primary screening, the crude extracts which displayed zone of inhibition more than 15 mm in diameter were selected for MIC studies. Each extract was evaporated at room temperature to dry residues and then using fresh solvent, different concentrations viz.10mg/ml, 5mg/ml, 1mg/ml and 0.5mg/ml were prepared. MIC was conducted with all concentrations by above mentioned disc diffusion method.

**Bioactivity Guided Fractionation**:
**Acetone Extract:** The extract was evaporated to dryness. Then chloroform, petroleum ether, ethyl acetate, acetone, ethanol, methanol and water were added successively with centrifugation at 3000 rpm for 20 minutes. All the fractions of solvent filtrates separated and kept in airtight borosil bottles at 4°C and tested by disc diffusion method.

**Aqueous extract:** The water extract was evaporated to dryness. Then chloroform, petroleum ether, ethyl acetate, acetone, ethanol methanol and water were added successively with centrifugation at 3000 rpm for 20 minutes. All the fractions of solvent filtrates separated and kept in airtight borosil bottles at 4°C and tested by disc diffusion method.

## 5. Result

**Antimicrobial assay:** *In vitro* antimicrobial activity is summarized in **Table-I.** Overall all bark extracts exhibited zone of inhibition ranging between 12mm - 22mm against sensitive pathogenic strains. Comparatively less activity was noted by ethyl acetate. Maximum inhibitory activity was shown with zone of inhibition exhibited by acetone extract against *K. pneumoneae* (22mm) followed by 21 mm zone of inhibition against *C. diphtheriae* by aqueous extract. Furthermore, it was observed that *B.subtillis*, *S. paratyphi-A*, *S. paratyphi-B* and *Ps. aeruginosa* were highly resistant against all test extracts. MIC of acetone extract revealed antimicrobial activity at 5 mg/ml against sensitive strains and by aqueous extract and at 1 mg/ml against susceptible bacteria.

Bioactivity Guided Fractionation indicated that active principles are dissolved in polar solvents.

## 6. Discussion

This investigation by primary screening reveals that active principals are dissolved in various solvent with different potency. Higher degree of potent bioactive phytochemicals is in acetone and in aqueous extracts. It was noted that in hot acetone extract of leaves *S. apetala* showed active principles are present. **[16].** Other researchers **[19]; [20]; [23]** noted that extracts made in organic solvents such as methanol, ethanol, ethyl acetate, chloroform and water of various parts of *S. alba* were active against *S. aureus*, *B. cereus*, *E. coli* and *Ps. aeruginosa*. Similar findings were also noticed for various mangrove species. **[14]; [15]; [16].** These earlier observations support the present study which indicates mangroves are loaded with antimicrobials which are dissolved in organic solvents. Bioactivity Guided Fractionation indicated that active constituents are dissolved in polar solvents. Similar findings were noted in activity guided fractionation of other mangrove plants occurring along Mumbai coast. **[14]; [15]; [24]; [18]**. These findings are in agreement with the present investigations.

## 7. Conclusion

This investigation supports that bioactive principles present in *S. alba* are active against sensitive gram positive and gram negative bacteria. It also reveals that mangrove species contains methanol, ethanol, acetone and water soluble antimicrobials. Further attempts may be made to isolate active principals which can be use in drug development.

## 8. Future Scope

It is suggested that the entire plant may be further explored for their active ingredients and further investigation is needed for its chemical composition in view of its pharmaceutical importance. Therefore it is suggested that methanol, ethanol, acetone and water can be use to extract bioactive principles from the mangrove species *S. alba*.

**Table 1:** Primary Screening of crude bark extracts of *S. alba* (Inhibition Zones in mm)

| | *B.subt* | *S.pyo* | *S.a* | *K.p* | *C.d* | *E.coli* | *S.t* | *Sp.A* | *Sp.B* | *Ps.aeru* |
|---|---|---|---|---|---|---|---|---|---|---|
| Acetone | - | 18 | 15 | 22 | - | - | 16 | - | - | - |
| Ethyl acetate | - | 13 | 15 | 15 | 14 | - | 15 | - | - | - |
| Water | - | 13 | - | 22 | 21 | 12 | - | - | - | - |

**Keywords:** - No zone of inhibition.

**Table 2:** MIC of potent extracts

| Solvent | Pathogen | Concentration mg/ml. | | | |
|---|---|---|---|---|---|
| | | 10 | 5 | 1 | 0.5 |
| Acetone | *S.pyo* | 20 | 15 | - | - |
| | *S.a* | 18 | 14 | - | - |
| | *K.p* | 17 | 12 | - | - |
| | *S.t* | 17 | 12 | - | - |
| Ethyl acetate | *S.a* | 15 | - | - | - |
| | *K.p* | 14 | - | - | - |
| | *S.t* | 14 | - | - | - |
| Water | *K.p* | 26 | 19 | 13 | - |
| | *C.d* | 26 | 18 | 13 | - |

**Keywords:** No zone of inhibition.

**Tables 3:** Activity Guided fractionation

| Extracts | Pathogens | Fractions | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | a | b | c | d | e | f | g | h |
| Acetone | *Kl. Pneumonia* | — | — | — | 12 | 13 | 13 | — | — |
| | *S.pyogens* | — | — | — | 13 | 12 | 13 | — | — |
| | *S.aureus* | — | — | — | 13 | 14 | 14 | — | — |
| | *S. typhi* | — | — | — | 11 | 12 | 13 | — | — |
| Water | *C.diphtheriae* | — | — | — | — | 13 | 13 | 15 | — |
| | *Kl. Pneumonia* | — | — | — | — | 12 | 11 | 13 | |

**Keywords:** —No zone inhibition, petroleum ether

a- chloroform
b- Petroleum ether
c- ethyl acetate
d- acetone
e- ethanol
f- methanol
g- water

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