

Antifungal Activity of *Avicennia Marina*

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Abstract- The genus *Avicennia* comprises eight species of mangrove trees that occur in intertidal zones of estuaries and seabeds found in tropical and temperate regions spanning throughout the world. The plants belonging to the genus have both ecological and economic benefits. Different parts of the plants have ethnomedicinal applications for treatment of various diseases such as cancer, diabetes, malaria, rheumatism, asthma, small pox and ulcer. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds. Further, scientific investigation and information of the therapeutic potential of the plant material is limited. Use of medicinal plants by man has been known for centuries and therapeutic efficacy of several herbal species has been widely described

Keywords- Mangroves, *Alternaria*, *Avicennia*, Solvent extract

I. INTRODUCTION

Recently, it has been strongly recommended that mangroves should be considered as a valuable source for chemical constituents with potential medicinal and agricultural values. Although the chemical constituents of most mangrove plants still have not been studied extensively, investigations have led so far to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents.

Study Area: Machilipatnam is between 16°10'N to 16.17°N latitudes and 81°09'E to 81.13°E longitudes on the southeast coast of India and in the east corner of Andhra Pradesh. Mangroves in this area lie between latitude 16° 0' - 16° 15'N latitude and 81° 10' - 81° 15' E longitude.

Description of genus *Avicennia* :

Avicennia marina (Avicenniaceae) has received some attention in determining its important chemical constituents. A member of the genus *Avicennia* are among the most salt tolerant mangroves and are often the first to colonize new deposits of sediment. The sap is salty and excess salt is

secreted through the leaves. The spreading root system provides stability in shifting substrates. There are vertical roots called pneumatophores projecting from the mud.

The flower, the largest among the *Avicennia* species has a diameter of 6 to 10 mm when expanded. It is orange yellow to lemon yellow in color. Action- Fruits are plastered on to boils and tumours, poultice of unripe seed stop inflammation, roots used for its aphrodisiac, bark is used to treat skin problems especially scabies, resin for snake bite and contraceptive by women, seed for ulcers

Important uses of *Avicennia*:

The tree is useful for preventing coastal erosion and as a windbreak

The tree is often a pioneer in sandy habitats, but may also invade mud flats

The tree tolerates heavy metals in the soil very well. The roots may be employed as a biological indicator of environmental exposure to copper, lead and zinc

Alternaria alternata is a fungus which has been recorded causing leaf spot and other diseases on over 380 host species of plant. It is a pathogen on numerous hosts causing leaf spots, rots and blights on many plant parts. It can be identified by Lacto phenol cotton blue staining method.

II. MATERIALS & METHODS

Plant material: The leaves of *Avicennia Marina* were collected from the mangrove forests of Gilakaladhindi, Mangrove Estuary, Krishna District.

Extract preparation: The amount of 100 grams of *Avicennia marina* leaves powder was added to 125 ml ethanol 96% or distilled water. The ethanolic and aqueous extracts mixture was preserved at laboratory temperature 25 °C for 48 hours and was stirred every few hours with a glass rod .

The collecting supernatant was centrifuged by 9000 rpm for 5-10 min. The supernatant was removed and reached to the original volume with ethanol or distilled water, then the samples packed in dark containers and stored at refrigerator temperature after filtered by 0.45 μ Whatman filter paper.

Potato Dextrose Agar (PDA) - Potato Dextrose Agar (PDA) is used for the cultivation of fungi. Potato Dextrose Agar (PDA) is a general purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products and testing cosmetics. PDA can be used for growing clinically significant yeast and molds. The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophytes.

Composition of Potato Dextrose Agar (PDA):

Potato infusion	50 gm
Dextrose	5 gm
Agar	5 gm
Distilled water	250ml
Commercial PDA Powder (20 gm dextrose, 15 gm agar, and 4 gm potato starch)	9.75 gm

III. STERILIZATION EQUIPMENT

Autoclave:

An **autoclave** is used to sterilize surgical equipment, laboratory instruments, pharmaceutical items, and other materials. It can sterilize solids, liquids, hollows, and instruments of various shapes and sizes. Autoclaves vary in size, shape and functionality. A very basic autoclave is similar to a pressure cooker; both use the power of steam to kill bacteria, spores and germs resistant to boiling water and powerful detergents.

Laminar Air Flow:

Laminar Air Flow has continuous displacement of air (it provides streamline flow of air) that passes through HEPA (High Efficiency Particulate Air) filter that removes the particulates from the air.

Laminar Air Flow are equipped with a UV lamp that should be turned on about 10-20 minutes before being used to sterilize the shell or cabinet or the surface of the Laminar Air Flow to avoid any kind of contaminations. Wipe down the surface with ethanol before and after each use.

Glassware & other chemical requirement also used.

Incubator:

An **incubator** is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO₂) and oxygen content of the atmosphere inside

IV. METHODOLOGY

Potato dextrose agar (PDA):

Potato dextrose agar medium is very commonly used for isolation of fungus as well as for its maintenance.

Procedure:

- Wash 50 g potato, peel off the skin, and slice them into small pieces.
- Cook the sliced potato in 250 ml. water for 10 minutes in an open vessel or pressure cooker for 10 minutes.
- Mix 5gms of agar with 250 ml. of water and boil in a cooker for 15 minutes.
- Collect the potato extract by filter through net filter.
- Add 5 gms of dextrose to the potato extract
- Mix thoroughly the molten agar with the potato- agar mixture and make the volume to 250ml with distilled water.
- Check the pH of the medium using pH papers.
- Pour the medium in to sterilized Petri dishes and plug with non- absorbent cotton wool.
- Arrange the tubes in a wire basket cover it with a waste paper sheet and tie tightly with a cotton thread.
- Sterilize them in an autoclave or a pressure cooker at 15 lbs pressure for 20 minutes.
- Take out the sterilized tubes after releasing the steam and keep them in a slanting position to get agar slants.
- After solidifying, the Petri plates are arranged in a wire basket and stored in a clean room for further use.
- Three Petri plates were with the plant extract and PDA Medium used and One petri plate was used as a Control.
- Spread plate technique was used for Fungi *Alternaria alternata* by inoculation under aseptic conditions in

Laminar air flow and incubated in Incubator for 24Hrs at 37°C.



fig-b (pouring media into petri plates)
fig-a(Preparation of media)

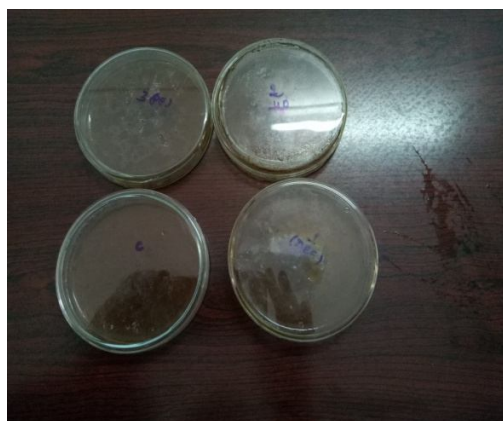


fig-c(Inoculation under aseptic conditions) fig-d(petri plates after incubation)

V. RESULTS & INTERPRETATION:

After 24Hrs of Incubation Fungal growth was observed in Control petri plate, Whereas, the other three petri plates with *Avicennia marina* plant extract did not show any growth of Fungi. This indicates that *Avicennia marina* has Anti fungal Property.

Phytochemical analysis:

Phytochemical analysis of *A. marina* plant extracts reveals the presence of tannins, sterols, flavonoids, iridoid glycosides and organic acids, but volatile oils, alkaloids and saponins were not detected in ethanol extracts. Phytochemical analysis using standard chemical reactions followed by subsequent separation on TLC silica-gel G plates supplemented with suitable detection methods revealed the production of more than four major spots of flavonoids, two spots of iridoid glycosides and two sterols. Alkaloids and saponins were not detected even though

VI. DISCUSSION

Recent strategies for discovering novel drugs from unexplored natural resources recommended marine plants as an important source of potentially useful chemicals (Harvey 2000). Biological activities and phytochemical screening are essential steps for exploration. Employing a wide range of bioassays (Khafagi et al. 2003) is valuable not only for preliminary biological characterization of new compounds, but also as essential guidance of the chemical isolation procedures (Claeson & Bohlim 1997). *Avicennia marina* is used to treat skin diseases in folk medicine, suggesting that it possess some natural antimicrobials. Therefore it was intended not only to test the antimicrobial activity of its extracts (which represents the constitutive activity) but also to test inducible activity

elicited by abiotic environmental stresses such as UV radiations (Ferreira & Duke 1997; Khafagi et al. 2001, 2003), and anti-bacteriophage and cytotoxic activities. In more than one investigation method was used.

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